ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 26 FEBRUARY, 1940 NUMBER 2

STUDIES ON THE MECHANISM OF DIABETIC DISTURBANCE USING THE "NITROGEN MINIMUM EXCRETION" AS A MEASURE OF GLYCONEOGENESIS[1,2]

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RUBNER (1) named the 'wear and tear quota' of protein (endogenous nitrogen metabolism) 'Abnutzungsquote.' This is measured by the amount of nitrogen excreted in the urine of an individual while on a protein-free diet. The diet must contain an excess of carbohydrate and fat (150 to 200% above the basal need) which supplies much more than the necessary amount of calories to prevent the breaking down of the cell protein.

The wear and tear quota of protein, expressed in nitrogen figures, is the amount of nitrogen derived from the breaking down of protein tissue. The amount of protein needed from the diet to furnish the protein required for the wear and tear quota is called the 'minimal nitrogen requirement' (2, 2a, 2b). Theoretically, the nitrogen figures for the wear and tear quota should be identical with those of the minimal nitrogen requirement. This estimate is true only in the case of children. In adults the amount of protein required for the minimal nitrogen requirement is higher than the amount of protein calculated from the Abnutzungsquote. The terms nitrogen minimum requirement, nitrogen minimum excretion and wear and tear quota of protein will be used interchangeably for practical purposes in this paper since the figures for all of these are almost identical. Under the conditions of the minimal nitrogen requirement we should expect a nitrogen equilibrium of the lowest possible amount of nitrogen. In adults, however, my former coworker Kraus (3) has shown that the nitrogen balance under the conditions of the minimal nitrogen requirement is slightly negative.

Friedrich von Mueller (4) and his pupils (5, 6, 7) carried out a series of experiments for the purpose of determining the nitrogen minimum excretion of individuals in normal health and disease. In these experiments it was shown that the nitrogen excretion could be related to body weight, to body surface, or to basal metabolism.

[1] This paper is written in honor of the eightieth birthday of my teacher, Friedrich von Mueller.

[2] This study was aided by grants from the Rockefeller Foundation, Bingham Associates Fund, and Charlton Fund.

It was demonstrated by Kraus (3) in a long series of experiments that the nitrogen minimum excretion should be related to body surface and not to body weight.

PROBLEM

Experiments reported in the literature concerning glyconeogenesis, that is, the new formation of carbohydrate from protein in patients with diabetes, were carried out in nitrogen equilibrium with high protein diets. The dextrose-nitrogen ratio (D:N) was also used in studying glyconeogenesis, estimating the amount of dextrose excreted above the amount given in the diet in relation to the nitrogen excretion (8–11).

The dextrose-nitrogen ratio, though helpful in judging the severity of the disease, is not a satisfactory measure of glyconeogenesis. It could lead to erroneous conclusions because the amounts of glucose utilized and excreted as well as the amounts of nitrogen excreted by a patient with diabetes changed daily despite the use of a standardized diet.

Many authors, among which are Geelmuyden, Laufberger, and Noorden and Isaac (12, 13, 14), suggest that increased glyconeogenesis is a primary factor in the cause of diabetes mellitus, while others attribute to it only a secondary rôle in the belief that the etiology of diabetes is a default in the disintegration of sugar in the organism.

In this study on patients with diabetes, nitrogen minimum excretion is used as a more conclusive means of measuring glyconeogenesis than nitrogen equilibrium. The inconvenience to the patient with diabetes, the antipathy aroused, and the difficulty in constructing an edible nitrogen-minimum diet consisting only of carbohydrate and fat have probably frustrated such an attempt heretofore. It was also impossible in the pre-insulin era to keep a severe diabetic constantly free of glycosuria on a diet high in carbohydrate and fat. It is possible at present, with the use of insulin, to control a patient with severe diabetes on a nitrogen minimum diet for several weeks.

The measurement of glyconeogenesis by the nitrogen minimum excretion in patients with diabetes, treated with or without insulin, should indicate which of the above theories is valid. Experiments on these patients with irradiation of the pituitary gland and injections of extracts of the pituitary gland and adrenal cortex were initiated to secure data concerning the effect of these glands on glyconeogenesis.

The patients with diabetes were selected for this study from the Diabetic Clinic, a division of the Medical Clinic of The Boston Dispensary. They had been controlled with insulin for some time previous to their selection for this study on a diet which was low in calories and ranged from 150 to 200 gm. of carbohydrate, 50 to 80 gm. of protein, and 70 to 80 gm. of fat.

METHODS OF PROCEDURE AND COMPOSITION OF THE NITROGEN MINIMUM DIET[3]

In this study the determinations of nitrogen excretion in the feces were omitted as the amounts are constant on a diet consisting almost entirely of carbohydrate and fat. The nitrogen excretion in the urine was related to the body surface and the body weight, as is customary in nitrogen minimum excretion studies.

The diets prescribed in the Diabetic Clinic were already low normal in protein content and therefore only 5 to 10 days were required to adjust these patients to yield a minimum nitrogen excretion on the nitrogen minimum diet. The nitrogen minimum diet requires at least twice the amount of calories as the basal caloric re-

[3] I am indebted to Miss Frances Stern, Chief of the Food Clinic of the Boston Dispensary, to Miss Helen Finkelstein, Associate in the Food Clinic, formerly on the Diagnostic Hospital staff, and Miss Elizabeth Kingsbury, dietitian in the Diagnostic Hospital.

quirement of the normal patient. The total calories in the diet are supplied primarily from carbohydrate and fat. The protein is limited to 8 or 9 gm. The following example illustrates a patient's daily diet.

Example. Sex: Male. Age: 18. Ht.: 174.6 cm. Av. Wt.: 66 Kg
Basal caloric requirement: 1625 Calories
Diet: Carbohydrate 291.5 Protein 9.0 Fat 271.5 Calories 3645.5

Food	Average household measure	Grams				Calories
		Total	C	P	F	
Butter	8 tablespoons	120			104.0	936.0
Cream 40%	⅜ cup	90	3.0	2.5	34.0	328.0
Ice cream	1 sm. scoop	75	15.0	3.0	10.5	166.5
Olives	40 large	400	40.0	tr.	120.0	1240.0
Uneedas	6	36	27.0	3.0	3.0	147.0
Pineapple, canned	2 slices	200	25.0	0.5		102.0
Apple sauce	2 small dishes	200	48.0			192.0
Jelly	6 teaspoons	90	90.0			360.0
Ginger ale	2¼ cups	540	43.5			174.0
Total			291.5	9.0	271.5	3645.5

The carbohydrate for the day in the nitrogen minimum diet is divided so that approximately one-third of the total carbohydrate is given at each meal. A typical day's food intake was as follows:

Morning	*Noon*	2:00 P.M.	*Night*	7:00 P.M.
Canned pineapple	Olives	Ginger ale	Olives	Ginger ale
Uneedas	Uneedas		Uneedas	
Butter	Butter		Butter	
Jelly	Jelly		Jelly	
Cream (H)	Cream (H)		Cream (H)	
Tea	Ice Cream (Vanilla)		Apple sauce	
	Tea		Tea	

The fluid intake is not restricted in amount but is kept at a constant level. This level is determined from the total amount of fluid in the actual diet plus the amount of water given at the request of the patient during the first day of the experiment.

The following table shows the relation of the nitrogen excretion both to the body surface (Meeh's and Dubois' formulas) and to the body weight in normal persons.

TABLE 1. NITROGEN MINIMUM EXCRETION OF NORMAL PERSONS IN RELATION TO BODY SURFACE AND TO BODY WEIGHT (KRAUS 3)

Weight Kg.	Height	Age	Sex	Total N output in urine	N output kg.	N/sq.m. (Meeh)	N/sq.m. (Dubois)	Caloric intake. % of requirement
10.8		2½	M	0.500	0.0463	0.9654	1.0023	194
39.9	155	13	M	1.224	0.0315	0.927	0.9872	284
61.0	160	25	F	2.199	0.035	1.133	1.3777	194
66.6	175	24	M	2.594	0.039	1.2835	1.4669	235
66.0	167	33	F	2.197	0.033	1.0937	1.2608	217
57.6	153	58	F	2.007	0.0348	1.094	1.3004	208
46.2	153.5	72	F	1.739	0.037	1.092	1.2963	242

CASE HISTORIES

The case histories of the following patients include the data taken from the rec-

ords of the Diabetic Clinic[4] of The Boston Dispensary and the Diagnostic Hospital. The charts are tabulations of the data concerning the patients during their study in the hospital. The following numbers of cases were studied: moderate diabetes 5, severe diabetes 3, renal glycosuria 2.

Case 1 (table 2) *E.M.*, a female, 15 years of age, was attending the second year of high school. Her diabetes was discovered in June 1936 when she was hospitalized for diabetic coma. She was admitted to the Boston Dispensary Diabetic Clinic in July 1936 and was given a diet of 250 gm. carbohydrate, 100 gm. protein, 70 gm. fat, 2030 calories, and 5 U of regular insulin before breakfast.

Previous to her admission to the Diagnostic Hospital in July 1937 she was on the same diet and her regular insulin was increased to 12 U before breakfast, 10 U before dinner, 10 U before supper, a total of 32 U of insulin. The fasting blood sugar and total cholesterol were not studied.

At the present time in the Diabetic Clinic, her diet is 175 gm. carbohydrate, 75 gm. protein, 70 gm. fat, 1650 calories, and she is taking 36 U of protamine zinc insulin before breakfast.

TABLE 2. [*E.M.*, female, age 13, height 153.67 cm.; basal caloric requirement, 1075; diet, 2900 calories=281% of requirement (C 380, P 10, F 150.)]

Weight, kg.	Days of period	Total urinary N, gm. (ave. for period)	N output/kg. Wt., gm. (ave. for period)	N/sq.m. Meeh formula (ave. for period)	N/sq.m. DuBois formula (ave. for period)	Insulin daily	Glycosuria, gm. (total for period)	Special procedures
				Period of adjustment				
43.3	2	2.93	0.0677	1.9312	2.1376	24 Prot. Ins. 16 Ins.	5	
43.37	4	1.98	0.0456	1.3028	1.4428	24 Prot. Ins. 16 Ins.	None	
				Nitrogen minimum period				
43.58	3	1.76	0.04038	1.1543	1.3384	24 Prot. Ins. 16 Ins.	None	
43.39	3	1.62	0.03733	1.0656	1.1803	24 Prot. Ins. 16 Ins.	None	6 cc. total pituitary extract
43.31	1	1.52	0.03508	1.0009	1.1082	None	4	None
43.18	4	1.81	0.04191	1.1944	1.3214	24 Prot. Ins. 16 Ins.	None	

Case 2 (table 3) *S.L.* was a female, 68 years of age, housewife, in whom diabetes was discovered by a private physician in May 1935. She was admitted to the Boston Dispensary Diabetic Clinic in November 1933. Her diet was 200 gm. carbohydrate, 75 gm. protein, 100

TABLE 3. [*S.L.*, age 68, height 156.55 cm.; basal caloric requirement, 1450; diet, 3520 calories=242% of requirement (C 230, P 9, F 245).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. for period)	N output kg. Wt., gm. (ave. for period)	N Sq. m. Meeh formula (ave. for period)	N Sq. m. DuBois formula (ave. for period)	Insulin daily	Glycosuria, gm. (total for period)
			Period of adjustment				
58.4	4	3.11	0.0532	1.6712	1.9706	14-10-14	14.65
			Nitrogen minimum period				
57.3	4	1.716	0.0299	0.9375	1.09522	12-12-14	None
38.9	5	2.087	0.0354	1.1198	1.3174	None	16.08 (4 days)
59.4	4	1.538	0.0258	0.82039	0.96729	14-12-14	None

[4] Caloric content of the diets prescribed was near or slightly above the basal requirement since it was realized that the patient usually eats somewhat above the diet prescribed.

gm. fat; 1900 calories. She was given 2 injections of regular insulin, 10 U before breakfast and 10 U before supper, a total of 20 U. Her complaints were polyuria and polydipsia. In addition to diabetes, a diagnosis of arthritis and hypertension was made.

Previous to her admission to the Diagnostic Hospital in 1935 her diet was 150 gm. carbohydrate, 70 gm. protein, 65 gm. fat; 1486 calories. She was controlled on 2 injections of regular insulin, 14 U before breakfast and 14 U before supper, a total of 28 U. The fasting blood sugar was 246 mg. and total cholesterol 248 mg. per 100 cc. of blood.

At the present time in the Diabetic Clinic, 3 years after the study, her diet is the same and she is controlled on 40 U of protamine zinc insulin before breakfast.

Case 3 (table 4) *A.L.*, a female, 50 years of age, was a counter girl at an ice cream stand. She discovered sugar in her urine in 1931 at which time she took the same diet and insulin as her brother who was under a physician's treatment. She was admitted to the Boston Dispensary Diabetic Clinic in 1934, where she was given a diet of 175 gm. carbohydrate, 75 gm. protein, 60 gm. fat; 1540 calories. She was given 3 injections of regular insulin, 24 U before breakfast, 16 U before dinner, and 16 before supper, a total of 56 U. Her complaints at that time were paresthesia, poor vision, polyuria, polydipsia and polyphagia.

TABLE 4. [*A.L.*, female, age 50, height 157.48 cm.; basal caloric requirement, 1575; diet, 3625 calories=231% of requirement (C 290, P 9, F 270).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. for period)	N output/kg. Wt., gm. (ave. for period)	N/sq.m. Meeh formula (ave. for period)	N/sq.m. DuBois formula (ave. for period)	Insulin daily	Glycosuria, gm. (total for period)
			Period of adjustment				
62.8	6	4.21	0.06704	2.1636	2.5746	24-16-10-6	None
			Nitrogen minimum period				
63.4	4	2.217	0.03590	1.1629	1.3871	22-16-4	None
62.7	4	2.75	0.04385	1.4151	1.6339	None	9.26 (2 days)
							None (2 days)

Previous to her admission to the Diagnostic Hospital in October 1935, her diet was 145 gm. carbohydrate, 70 gm. protein, 50 gm. fat; 1310 calories. She was controlled on 3 injections of regular insulin, 24 U before breakfast, 16 U before dinner, and 16 U before supper, a total of 56 U. The fasting blood sugar was 298 mg. and total cholesterol 228 mg. per 100 cc. of blood.

At the present time in the Diabetic Clinic, 3 years after the study, her diet is 135 mg. carbohydrate, 75 gm. protein, 70 gm. fat; 1420 calories. She is controlled on 56 U of protamine zinc insulin before breakfast.

Case 4 (table 5) *A.S.* was a charwoman, 65 years of age. Diabetes was discovered in the Boston Dispensary Medical Clinic and she was admitted to the Diabetic Clinic. At that time, July 1932, she complained of polyuria, polydipsia and pruritis. She was then given a diet of 125 gm. carbohydrate, 75 gm. protein, 50 gm. fat; 1250 calories. Previous to her admission to the Diagnostic Hospital in July 1935, her diet was 150 gm. carbohydrate, 75 gm. protein, 50 gm. fat; 1350 calories. She was controlled on 3 injections of regular insulin, 18 U before breakfast, 10 U before dinner and 18 U before supper, a total of 46 U.

When last seen in the Diabetic Clinic in August 1935, the patient was on a diet of 150 gm. carbohydrate, 75 gm. protein, 50 gm. fat; 1350 calories. She was fairly well controlled with 2 injections of insulin, 18 U before breakfast and 18 U before supper, a total of 36 U.

Case 5 (table 6) *J.McK.*, was an unemployed male, 64 years of age. Diabetes was discovered in July 1928 in the Boston Dispensary where the patient was admitted in acidosis. The diet prescribed was 100 gm. carbohydrate, 75 gm. protein, 160 gm. fat; 2160 calories. He was given 3 injections of regular insulin, 16 U before breakfast, 16 U before supper and 6 U before bed-time, a total of 38 U. He complained of sore feet at that time. In addition to diabetes, the following diagnoses were made: varicose veins, peripheral sclerosis and diabetic neuritis.

TABLE 5. [A.S., female, age 73, height 158.8 cm.; basal caloric requirement, 1625; diet, 3600 calories=221% of requirement (C 283, P 9, F 271).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. for period)	N output/kg. Wt., gm. (ave. for period)	N/sq. m. Meeh formula (ave. for period)	N/sq. m. DuBois formula (ave. for period)	Insulin daily	Glycosuria, gm. (total for period)	
				Period of adjustment				
65.4	6	6.29	0.0962	3.1464	3.757	20-14-14-10	None	
				Nitrogen minimum period				
65.4	4	2.217	0.0337	1.1061	1.3213	20-14-14-10	None	
65.4	6	2.031	0.0310	1.0155	1.2128	20-14-14-10	None	X-ray to pituitary daily
65.4	2	1.925	0.0297	0.9696	1.1550	20-14-14-10	None	
65.4	5	2.388	0.03708	1.2073	1.4361	None	None	

Previous to his admission to the Diagnostic Hospital in September 1935, his diet was 150 gm. carbohydrate, 75 gm. protein, 50 gm. fat; 1324 calories. He was contolled on 3 injections of regular insulin, 20 U before breakfast, 8 U before dinner and 12 U before supper, a total of 40 U. The fasting blood sugar was 440 mg. and total cholesterol 198 mg. per 100 cc. of blood.

Two years after the study in the Diabetic Clinic, the patient was on a diet of 150 gm. carbohydrate, 70 gm. protein, 70 gm. fat; and 1510 calories. He was controlled on 24 U of protamine zinc insulin. The patient died in June 1938.

TABLE 6. [J. McK., male, age 65, height 175.26 cm.; basal caloric requirement, 1950; diet, 3625 calories=185% of requirement (C 315, P 8, F 260).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. for period)	N output/kg. wt., gm. (ave. for period)	N/sq. m. Meeh formula (ave. for period)	N/sq. m. DuBois formula (ave. for period)	Insulin daily	Glycosuria, gm. (total for period)	Remarks
				Period of adjustment				
78	7	4.03	0.0516	1.7927	2.0801	10-10-20-20 (1 day) 20- 8-10-10 (3 days) 25-10-14 (3 days)	23.22	
				Nitrogen minimum period				
80	4	1.727	0.02150	0.7537	0.8806	30-10-4	5.91	
78	6	3.46	0.045	1.5713	1.8095	None	6.92	Acetone vomiting

Case 6 (table 7) *A.B.*, was a male, 21 years of age, unemployed. Diabetes was discovered in February 1934 and he was treated in a Boston hospital for diabetic coma and from there referred to the Out-Patient Department of the Boston Dispensary. On admission to the Boston Dispensary Diabetic Clinic in March 1934, he was given a diet of 220 gm. carbohydrate, 85 gm. protein, 100 gm. fat; 2120 calories. Regular insulin was given in 3 injections, 10 U before breakfast, 10 U before dinner and 10 U before supper, a total of 30 U. At this time the patient complained of polyuria, polydipsia and polyphagia.

Previous to his admission to the Diagnostic Hospital in June 1935, his diet was the same and he was taking 18 U of regular insulin before breakfast and 14 U of regular insulin before supper, a total of 32 U. Three hours after eating the blood sugar was 224 mg. and total cholesterol 198 mg. per 100 cc. of blood. During the course of treatment at the Boston Dispensary he was hospitalized once for acidosis caused by a carbuncle.

In the Diagnostic Hospital, fasting blood sugar determinations made at one hour intervals were: 9 A.M., 139.8 mg.%; 10 A.M., 113.6; 11 A.M., 109.3; 12 noon, 109.3; 1 P.M., 106.5 mg.%.

At the present time in the Diabetic Clinic, 3 years after the study, his diet is 205 gm. carbohydrate, 80 gm. protein, 65 gm. fat; 1701 calories. He is controlled on 50 u protamine zinc insulin before breakfast.

TABLE 7a. [A.B., male, age 18, height 174.6 cm.; basal caloric requirement, 1625; diet, 3625 calories=223% of requirement (C 290, P 9, F 270).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. of period)	N output/kg. wt., gm. (ave. of period)	N/sq. m. Meeh formula (ave. of period)	N/sq. m. DuBois formula (ave. of period)	Insulin daily	Glycosuria, gm. (total in period)	Special procedures
Period of adjustment								
65.5	6	11.07	0.1670	5.5322	6.1695	18-18-10-6	98.91	
65.3	3	4.07	0.0619	2.0367	2.2703	16-16-10-6	None	
Nitrogen minimum period								
65.52	4	4.235	0.0646	2.1164	2.3604	14-14-10-6	None	
65.53	6	3.708	0.05658	1.8526	2.0665	10-12-8-8	None	X-ray pituitary days, 1200 r.
Second nitrogen minimum period								
67.02	3	4.13	0.0616	2.0331	2.2799	10-12-8-8		
66.19	3	6.15	0.092914	3.0527	3.4129	None	105.61 (3 day total)	
65.39	3	4.32	0.066065	2.1618	2.4098	10-12-8-8	70.12 (1 day after)	
Third nitrogen minimum period (After one year—10/29/36)								
66.79	4	3.592	0.05378	1.7885	1.9687	22-18-10-6	None	
66.79	5	3.621	0.0550	1.8051	2.0152	22-14-10-6	None	4 cc. Eschatin daily
66.79	5	3.413	0.05194	1.7023	1.8999	22-14-10-6	None	4 cc. Pituitary extract daily

Case 7 (table 8) *L.B.*, a male, 28 years of age, is a musician with very irregular hours and activity. His diabetes was discovered in 1929 by a private physician and he was given a diet and insulin. He was admitted to the Boston Dispensary Diabetic Clinic in August 1934 where he was given a diet of 150 gm. carbohydrate, 75 gm. protein, 100 gm. fat; 1900 calories. He was given 14 u of regular insulin before breakfast. His complaints at that time were polyuria, polydipsia, polyphagia and paresthesia. In addition to diabetes, a diagnosis of oral sepsis and myopia was made.

Previous to his admission to the Diagnostic Hospital for study, his diet was 150 gm. carbohydrate, 65 gm. protein, 75 gm. fat; 1535 calories. He was controlled with 3 injections of regular insulin, 12 u before breakfast. 8 u before supper and 6 u before bed-time, a total of

TABLE 8. [L.B., male, age 28, height 163.6 cm.; basal caloric requirement, 1560; diet, 3700 calories=239% of requirement (C 300, P 9, F 275).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. for period)	N output/kg. wt., gm. (ave. for period)	N/sq. m. Meeh formula (ave. for period)	N/sq. m. DuBois formula (ave. for period)	Insulin daily	Glycosuria, gm. (total for period)
Period of adjustment							
62.5	8	5.06	0.0809	2.6079	3.0201	22-6-16-10 (3 days) 14-6-16-10 (5 days)	None
Nitrogen minimum period							
61.3	4	3.96	0.0696	2.0711	2.3862	14-6-11	None
59.7	4	5.52	0.0924	2.935	3.3609	None	328.83
58.5	7	3.15	0.0537	1.691	1.9336	14-6-11	14 (5 days) None (2 days)

26 U. The fasting blood sugar was 264 mg. and total cholesterol 185 mg. per 100 cc. of blood. Once during his treatment at the Boston Dispensary he had acidosis as a result of an infection.

At the present time in the Diabetic Clinic, after the study, he is on the same diet and is controlled with 28 U of protamine zinc insulin before breakfast.

Case 8 (table 9) M.W., a female, 30 years of age, was formerly a waitress, but at the present time, is unemployed. Her diabetes was discovered in a hospital in Boston where she was treated for diabetic coma in 1931. In 1933 she was treated again for diabetic coma in the same hospital and from there referred to the Boston Dispensary Diabetic Clinic. Her diet was 135 gm. carbohydrate, 67 gm. protein, 90 gm. fat; 1618 calories. She was given 25 U of regular insulin before breakfast, 5 U before dinner, 25 U before supper and 2 U before bedtime, a total of 57 U. She was admitted to the Boston Dispensary Diabetic Clinic in September 1933 where she was given a diet of 200 gm. carbohydrate, 75 gm. protein, 100 gm. fat; 2000 calories. She was given 3 injections of regular insulin, 20 U before breakfast, 20 U before dinner, 20 U before supper, a total of 60 U. Her complaints at that time were polyuria, polydipsia and paresthesia.

TABLE 9. [M.W., female, age 28, height 161.29 cm.; basal caloric requirement, 1425; diet, 3600 calories=255% of requirement (C 280, P 9, F 270).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. of period)	N output/kg. wt., gm. (ave. of period)	N/sq. m. Meeh formula (ave. of period)	N/sq. m. DuBois formula (ave. of period)	Insulin daily	Glycosuria, gm. (total for period)	Special procedures
				Period of adjustment				
57.5	3	4.13	0.0815	2.2563	2.5775	20-14-14-10	2.52	
				Nitrogen minimum period				
59.2	4	2.61	0.044122	1.3967	1.6102	20-14-12-6	None	
57.5	2	5.69	0.09887	3.1005	3.5498	None	137.65	
57.4	3	2.83	0.0496	1.5528	1.77406	20-16-12-4	135.89	
58.2	6	2.25	0.038191	1.2211	1.4019	20-16-12-4	36.04	X-ray to pituitary 1200 r; 6 days

Previous to her admission to the Diagnostic Hospital in July 1935, her diet was 200 gm. carbohydrate, 80 gm. protein, 120 gm. fat; 2200 calories. She was controlled on 3 injections of regular insulin, 10 U before breakfast, 10 U before dinner, 20 U before supper, a total of 40 U. Blood sugar, 3½ hours after eating, was 168 mg. and total cholesterol was 191 mg. per 100 cc. of blood.

Fasting blood sugar determinations made at one hour intervals in the Diagnostic Hospital were: 9 A.M., 235 mg.%; 10 A.M., 250; 11 A.M., 251; 12 noon, 234; 1 P.M., 239 mg.%.

At the present time in the Diabetic Clinic, 3 years after the study, her diet is 180 gm. carbohydrate, 70 gm. protein, 90 gm. fat; 1810 calories. She is controlled on 52 U of protamine zinc insulin before breakfast.

Case 9 (table 10) A.F., was an unemployed male, 23 years of age. Glycosuria was discovered in February 1936 on routine examination in the Skin Clinic where he was being treated for a neisserian infection. He was admitted to the Boston Dispensary Diabetic Clinic in February 1936. Results of a glucose tolerance test were as follows.

	BLOOD SUGAR mg. per 100 cc.	URINE SUGAR gm. per 100 cc.
Fasting	85	1.7
1 hour	154	1.9
2 hours	82	1.4
3 hours	79	0.2

Urinalyses carried out for 5 of the patient's sisters showed that 3 had glycosuria and nor-

mal blood sugars, but no diabetic symptoms. A diagnosis of renal glycosuria was made and the patient was referred to the Diagnostic Hospital for study.

He was admitted to the Diagnostic Hospital in March 1937 and discharged in May 1937. Results of a fasting blood sugar curve taken at hourly intervals were: 9 A.M., 93 mg.%; 10 A.M., 87; 11 A.M., 85; 12 noon, 85; 1 P.M., 88 mg.%.

TABLE 10. [A.F., male, age 23, height 159.4 cm.; basal caloric requirement, 1400; diet, 3140 calories=224% of requirement (C 365, P 10, F 183).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. of period)	N output/kg. wt., gm. (ave. of period)	N/sq. m. Meeh formula (ave. of period)	N/sq. m. DuBois formula (ave. of period)	Insulin daily	Glycosuria, gm. (total of period)	
Period of adjustment								
56.07	4	5.99	0.10683	3.3211	3.8107	None	217 (blood sugar 91)	
Nitrogen minimum period								
55.45	4	3.40	0.06132	1.8991	2.1732	None	230	
55.12	4	3.42	0.06204	1.9178	2.1922	5-5	180	
54.72	4	2.57	0.04692	1.4473	1.65007	10-10-5	159	
54.23	4	3.05	0.0562	1.7290	1.9625	15-10-10	160	2×2 cc. total pituitary
54.11	4	3.92	0.0724	2.2242	2.5323	None	204	2×5 cc. total pituitary
53.78	7	3.66	0.0605	2.0866	2.3709	15-10-10	306	
53.3	4	3.14	0.0586	1.7994	2.0403	None	165	

A fasting blood sugar curve at hourly intervals with 1 cc. adrenalin were: 9 A.M., 97 mg.%; 10 A.M., 147; 11 A.M., 123; 12 noon, 99; 1 P.M., 90 mg.%. A fasting blood sugar curve at hourly intervals with 14 U of regular insulin were: 9 A.M., 82 mg.%; 10 A.M., 82; 11 A.M., 79; 12 noon, 58; 1 P.M., 54 mg.%. A fasting blood sugar curve at hourly intervals with 1 cc. of antuitrin-T (thyrotropic hormone) was: 9 A.M., 76 mg.%; 10 A.M, 73; 11 A.M., 80; 12 noon, 81; 1 P.M., 73 mg.%.

All samples of urine during these tests showed 2 to 5% glycosuria.

Case 10 (table 11) M.S., a female, 27 years of age, was a secretary with a regular schedule of living. Glycosuria was accidentally found in a physical examination. She complained of fatigue. There was no polyuria and no polydipsia.

She was admitted to the Boston Dispensary where diagnosis of renal diabetes was made. There, she was placed on a liberal diet with no restriction of carbohydrate. She had constant glycosuria of about 10 gm. a day which seemed to be independent of the sugar intake. She had received no insulin previously. While she was in the hospital, she was put on a nitrogen-minimum diet.

TABLE 11. [M.S., female, age 27, height 159.4 cm.; basal caloric requirement, 1100; diet, 3735 calories=340% of requirement (C 314, P 9, F 271).]

Weight, kg.	Days of period	Total urinary N, gm. (ave. for period)	N output/kg. wt., gm. (ave. period)	N/sq. m. Meeh formula (ave. for period)	N/sq. m. DuBois formula (ave. for period)	Insulin daily	Glycosuria, gm. (total for period)
Period of adjustment							
44	4	3.44	0.0782	2.2417	2.4259	4-4-4-2	55.08
Nitrogen minimum period							
44.5	5	2.51	0.0574	1.6235	1.7616	5-5-5	41.13

Her basal metabolic rate was +18 to +24. A fasting blood sugar curve taken at hourly intervals was: 9:30 A.M., 81 mg.%; 10:30 A.M., 76; 11:30 A.M., 81; 12:30 P.M., 80; 1:30 P.M., 83 mg.%.

During our study, she was never sugar free. Insulin did not influence her sugar output markedly.

RESULTS

The data obtained from this investigation on glyconeogenesis in patients with diabetes, on a nitrogen minimum diet, brought out many unexpected findings.

A. Patients with moderate and severe diabetes could be controlled on a nitrogen minimum diet containing 100% more calories than the basal caloric requirement, consisting of approximately 300 gm. of carbohydrate, 275 gm. of fat and 8 to 9 gm. of protein.

B. The amount of insulin required was no greater on this diet than was previously used in the Diabetic Clinic.

C. There was no gain in weight in these patients, for the most part at bed rest, despite the high caloric content of the nitrogen minimum diet.[5]

D. The nitrogen minimum excretion figures on the patients with moderate diabetes (*cases 1, 2, 3, 4, 5*) obtained after the period of adjustment, were the same as those obtained from normal persons. The nitrogen minimum excretion figures still remained within normal limits after omission of insulin in 3 of these cases. The nitrogen minimum excretion figures rose only slightly after omission of insulin in 2 patients (*cases 2, 5*), despite the fact that sugar was excreted in the urine .

E. The nitrogen minimum excretion figures on patients with severe diabetes (*cases 6, 7, 8*), all of whom had at some time been in precomatous or comatous condition, were higher than in normal individuals. This included the actual figures as well as those related to body surface or body weight. The nitrogen minimum excretion figures increased 100% to 150% with the omission of insulin, a sign of glyconeogenesis from tissue protein. In *case 6*, after omission of insulin for 3 days, 105 gm. of sugar was excreted in the urine and during this period the excretion of nitrogen increased to 6.06 gm., equivalent to 37.8 gm. of protein. If the total amount of 37.8 gm. of protein were converted into sugar in intermediary metabolism, about 68 gm. of excreted sugar still remains unaccounted for by glyconeogenesis. In *case 7*, during the 4 days in which insulin was omitted, 328 gm. of sugar was excreted in the urine and the nitrogen excretion increased to 6.25 gm. or 39 gm. of protein. About 289 gm. of sugar was excreted which was not produced by glyconeogenesis. In *case 8*, after omitting insulin for 2 days, it was found that there was an excretion of 137.67 gm. of sugar in the urine while nitrogen excretion increased to 6.18 gm. equivalent to 38.61 gm. of protein. About 99 gm. of sugar was excreted which were not produced by glyconeogenesis.

F. Two patients (*cases 9, 10*) with renal glycosuria were studied. One of these (*case 9*), with daily glycosuria of 40 gm. was slightly influenced by the use of insulin. The daily excretion of the other patient (*case 10*) was also slightly influenced by insulin. The nitrogen minimum excretion in both these patients, despite the use of insulin, was definitely increased. Neither of them, however, was free of glycosuria during the regime on the nitrogen minimum diet.

G. The pituitary gland in 3 patients (*cases 4, 6, 8*) was irradiated on 6 consecutive days. The irradiation of the pituitary gland resulted in a slight decrease of the nitrogen minimum excretion in all 3 patients. This decrease in the nitrogen minimum

[5] These findings will be investigated further in a special study on obese patients.

excretion was only transitory during the irradiation period. The condition of the diabetes of these patients was not improved for any length of time.

H. In patients with diabetes (*cases* 3, 6), the daily injection over a period of days of 4 cc. total pituitary extract as well as 4 cc. eschatin had no influence on the nitrogen minimum excretion and consequently no effect on glyconeogenesis. This is not to be considered as conclusive since the hormones were not given over a sufficiently long period of time and since the pharmaceutical preparations may not have been adequate in dosage or concentration for this experiment. It may be of some significance that the injection of total pituitary extract increased the already high nitrogen minimum excretion in one of the patients with renal diabetes (*case* 8), in contrast to the ineffectiveness of the same extract on the nitrogen minimum excretion of patients with diabetes (*cases* 3, 6).

DISCUSSION

On the supposition that an increased glyconeogenesis in patients with diabetes is the primary reason for the metabolic disturbance, an increased nitrogen minimum excretion should be anticipated. The normal findings in patients with moderate diabetes who are controlled with insulin are obviously in disagreement with the theory of increased glyconeogenesis as the etiological factor in diabetes. The definite increase in nitrogen minimum excretion in patients with severe diabetes who are controlled with insulin may be explained as increased glyconeogenesis despite the fact that sugar was not excreted in the urine during this time. The increased nitrogen minimum excretion as a result of the omission of insulin in these cases is a sign of glyconeogenesis from tissue protein. It is evident, having determined the amount of glyconeogenesis from the increased nitrogen excretion, that the amount of sugar excreted in the urine during this period is 4 to 7 times higher than the amount of glyconeogenesis anticipated. The calculations on the patients with severe diabetes (*cases* 6, 7) prove even more conclusively than do the nitrogen minimum excretion figures on patients with moderate diabetes (*cases* 1 to 5) that the metabolic disturbance in diabetes is primarily not an increased glyconeogenesis. It has thus been demonstrated that sugar excreted by patients with severe diabetes is for the greater part the sugar from food intake which has not been utilized in intermediary metabolism. It is therefore evident that the primary disturbance in diabetes mellitus is to be found in a partial failure in the sugar molecule to be disintegrated in intermediary metabolism, and that glyconeogenesis is a result of and not a primary factor in the cause of diabetes.

In patients with moderate diabetes there was no increase in glyconeogenesis with and without insulin during the nitrogen minimum excretion period. In patients with severe diabetes the glyconeogenesis increased during the time the patient was controlled with insulin. When insulin was omitted, the glyconeogenesis increased still more. The amount of sugar excreted after omission of insulin did not exceed to a remarkably high degree the amount of glyconeogenesis. The figures of the nitrogen minimum excretion in relation to the amount of sugar excreted after the omission of insulin, offer an explanation for the effect of insulin. Insulin does not act directly on glyconeogenesis but affects the utilization of sugar in intermediary metabolism.

SUMMARY

A method has been described for studying glyconeogenesis and nitrogen minimum excretion on patients with diabetes on a nitrogen minimum diet.

The nitrogen minimum excretion was normal in 4 cases of moderate diabetes even with the temporary omission of insulin.

The nitrogen minimum excretion was slightly increased in 3 cases of severe diabetes. With the omission of insulin the nitrogen minimum excretion on these patients increased, and simultaneously large quantities of sugar, 4 to 7 times higher than the expected amount from the increased figures of the nitrogen minimum excretion, appeared in the urine. Insulin does not act directly on glyconeogenesis, but acts on the intermediary sugar disintegration as shown by the figures of the nitrogen minimum excretion, in relation to the quantity of sugar excreted in the urine.

Irradiation of the pituitary gland caused only a slight temporary depression on the nitrogen minimum excretion.

Injections of extracts of total pituitary and adrenal cortex had no influence on the nitrogen minimum excretion of patients with moderately severe diabetes. Two cases of renal diabetes showed a high nitrogen minimum excretion which was definitely increased after injections of total pituitary extract. This should not be considered conclusive since the hormones were not given over a sufficiently long period of time and since the preparations available at present may not be concentrated enough for such an experiment.

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ORAL USE OF PREGNENINONOL IN FUNCTIONAL MENO-METRORRHAGIA

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INHOFFEN AND HOHLWEG (1) prepared recently a derivative of estradiol, which was identified as pregnen-in-on-3-ol-17. These workers reported that, when this compound was injected into immature mice, previously treated with estrogens, it was approximately one-third as effective in producing progestational changes as was synthetic progesterone, similarly administered. It was observed, however, that when pregneninonol was given by mouth to similar animals, it was approximately 15 times as potent as progesterone given orally. A well developed progestational endometrium was produced in immature mice following the oral administration of 4 mg. of pregneninonol, whereas 60 mg. of progesterone by mouth were required for a similar response.

One of us (E.C.H., 2) reported previously that pregneninonol and certain estrogens had been given orally in the treatment of meno-metrorrhagia and that no untoward reactions or evidence of toxicity had followed. Apparent beneficial effects upon the character of flowing were described. The present communication reports these studies in detail.

METHOD

A group of young women with functional meno-metrorrhagia was selected for study. The irregular uterine bleeding of these patients was related to diverse grades of ovarian failure, as judged by physical findings, hormonal titrations and by studies of endometriotropic responses.

In view of the effective hemostatic-hemorrhagenic responses which one of us (E.C.H., 3) described from cyclic therapy which comprised injections of estrogens and progesterone, an attempt was made to duplicate this form of treatment, using orally certain estrogens and pregneninonol. Estradiol and estriol glucuronide were chosen as the estrogens for use. The recent studies of Smith and Smith (4), which indicated that estriol and pregnandiol (metabolic product of progesterone) were characteristic excretionary products of the progestational phase of the menstrual cycle, led us to give estriol glucuronide and pregneninonol simultaneously. Their observations, likewise, influenced us in the choice of estradiol as the estrogen for the preliminary part of the cyclic therapy. The schema of this therapy was the duplication of estradiol-estrone-estriol reaction described as being characteristic of estrogenic metabolism.

The therapeutic approach in general was as follows: the meno-metrorrhagia of these patients was controlled immediately by either curettage or by injections of estrogen and progesterone. Oral therapy was begun directly following curettage or

when cyclic bleeding had been secured from the injectional treatment. Estradiol[1] was given orally 4 times daily in individual doses of 600 oral units (0.5 mg.) for 14 days. This treatment was started, as a rule, at the onset of bleeding or a few days thereafter or immediately following curettage. Immediately following these 14 days of treatment, sodium estriol glucuronide as a powder[2] or estriol glucuronide in solution as liquid emmenin[3] was given orally once or 4 times a day in total daily doses which ranged from 3900 to 1800 day-oral units. Simultaneously the patient was given pregneninonol[4] orally 2 to 4 times daily in individual doses which varied from 10 to 40 mg. and in total daily doses which ranged from 40 to 160 mg. The estriol glucuronide-pregneninonol therapy lasted 10 to 14 days and was discontinued when bleeding began.

Observations upon the patients treated according to this system dealt primarily with the following responses.

(*a*) Clinical tolerance of the patients for these sterols, particularly pregneninonol.

(*b*) Cyclicity and quantitative characteristics of bleeding during therapy.

(*c*) Endometriotropic responses, judged by studies of endometrial biopsies taken at the onset of episodes of bleeding.

(*d*) Alterations in the urinary excretion of sodium pregnandiol glucuronide (i.e., investigation of possible metabolism of pregneninonol into the pregnandiol-complex): 24-hour specimens of urine were quantitated prior to therapy and during therapy by the method of Venning (5).

(*e*) Alterations in the urinary titers of androgens (i.e., investigation of the possible metabolism of pregneninonol into androgenic sterols): 24-hour specimens of urine were quantitated prior to therapy and during therapy by the colorimetric method of Oesting (6).

Two other groups of patients are being treated in a different manner. One group is receiving only the pregneninonol-portion of the cyclic therapy without the estriol glucuronide. The other group is being given the estradiol-estriol glucuronide portion of the oral therapy without pregneninonol being used. These 2 groups of patients will be reported in detail later.

ANALYSIS OF DATA

The observations upon 7 young women, whose ages varied from 17 to 30 years have progressed sufficiently to permit their being reported. Figures 1 and 2 present the data secured during these studies. The clinical records of these patients prior to the beginning of oral therapy are presented in the protocols.

Case 01, C.B. was a colored, single, 17-year-old girl who had had episodes of metrorrhagia for 2 years. A curettage was performed July 2, 1938 to check a depleting hemorrhage which had lasted for approximately 5 months. The endometrium was of estrogenic type. Following curettage the patient was given 5 series of cyclic therapy with sex sterols by injection according to the method described by one of us (E.C.H., 3). Cyclic and quantitatively normal bleeding followed each series. Endometriotropic responses were sampled by biopsy at the onset of an episode of flowing. The first 3 of these biopsies showed persistent estrogenic endometria (E), whereas the latter 2 showed mixed endometria (M). The details of this patient's treatment to this point, have been reported as *Case* 23 in another publication by our group (7). Following discontinuation of injections, the patient had another cyclic episode of bleeding. The endometrium at the onset of this bleeding was of mixed character (M).

[1] Estradiol (progynon-DH) was supplied by Schering Corporation. They estimated 0.5 mg. to yield 600 active oral units or 6000 R.U. by injection.

[2] This material was supplied by Ayerst, McKenna & Harrison as an incompletely purified powder. Two supplies were available: one which was estimated to contain 390,000 day-oral units per gm. of powder; another estimated to contain 190,000 day-oral units per gm. of powder.

[3] This material was supplied by Ayerst, McKenna & Harrison. Each cc. of liquid emmenin was said to contain 30 day-oral units.

[4] Pranone supplied by Schering Corporation.

During the previous 6 cycles, urinary titers of sodium pregnandiol glucuronide were determined. The total excretion per cycle was as follows.

1st cycle	42 mg.	4th cycle	25 mg.
2nd cycle	6 mg.	5th cycle	19 mg.
3rd cycle	41 mg.	6th cycle (No ℞)	15 mg.

Following the 6th cycle, a series of oral therapy with pregneninonol was given. Urinary values of pregnandiol-complex were titrated. The details of these are given in figure 1.

Case 02, *V.H.* was a white, married, 29-year-old woman, para 0-0-0, who had had episodes of amenorrhea and metrorrhagia since menarche at 13 years of age. Titrations of 24-hour specimens of urine for androgens and pregnandiol-complex were carried out from January 25, 1939 to February 9, inclusive. An episode of bleeding began February 9 and lasted until February 18. A biopsy of the endometrium taken at the onset of flowing showed a hyperestrogenic (E +) response.

Titrations of urine specimens were resumed February 20 and were continued until the onset of the next period, March 10. Cyclic therapy with sterols by injection (3) was begun February 16. Biopsy taken at onset of bleeding, showed a normal menstrual endometrium. Bleeding lasted from March 10 to 15 and was quantitatively normal.

Titrations of urine specimens were resumed March 24 and continued to April 2. Cyclic therapy by injection was given from March 25 to April 1. Bleeding began April 3 and lasted to April 7; it was quantitatively normal. No biopsy was taken.

(The record of this patient to this point was presented graphically in another report by our group, 8.)

The values for urinary hormonal titers during the 3 cycles described were as follows.

1st cycle (No. ℞):	
androgens, average daily excretion	26 I.U.
pregnandiol-complex, total	8.0 mg.
2nd cycle (℞):	
androgens, average daily excretion	12 I.U.
pregnandiol-complex, total	6 mg.
3rd cycle (℞):	
androgens, average daily excretion	10 I.U.
pregnandiol-complex, total	0 mg.

Oral therapy with estradiol, sodium estriol glucuronide and pregneninonol was started April 12. Titrations of urines for androgens and the pregnandiol-complex were made during two cycles of this therapy (fig. 1).

The details of oral therapy, hormonal titrations and bleeding are shown in figure 1.

Case 03, *C.H.* was a white, single, 27-year-old woman who had had metrorrhagia for 3 years. A curettage was performed May 12, 1938 to check an episode of metrorrhagia which had lasted 3 months. The endometrium was of estrogenic character. From the time of this curettage until the middle of December 1938, the patient received 8 series of cyclic therapy with sex sterols, (3) (a record of this patient's therapy to this point, was reported as *Case* XI, in another publication by our group, 7). Cyclic and quantitatively normal bleeding resulted. Endometrial biopsies taken at the onset of the 8 episodes of bleeding showed estrogenic responses in all but one instance, in which a mixed endometrium was observed. The patient then was allowed to go without treatment for 3 months. The result was 2 cyclic and quantitatively normal episodes of bleeding. The third episode of bleeding after therapy was prolonged. Cyclic therapy was started again and given one month with re-establishment of cyclic bleeding.

Oral therapy then was started, following a period of flowing which began April 5, 1939 and lasted until April 11 (fig. 1).

Case 04, *A.J.* was a white, married, 20-year-old woman, para 0-0-0, who had had menometrorrhagia since menarche 7 years previously. A curettage was performed October 21, 1938 to stop an episode of bleeding which had lasted 2 months. The endometrium was of a hyperestrogenic type (E +). Prolonged episodes of bleeding recurred in November and De-

cember. A series of cyclic therapy with sex sterols (3) by injection was given in January 1939. A quantitatively normal episode of bleeding began February 4 and lasted to February 7. Biopsy taken at the onset of bleeding showed a mixed endometrium (M). Three additional series of this therapy were followed by cyclic and quantitatively normal bleeding. Two of these episodes of bleeding were sampled by endometriotropic studies; in both instances endometria were persistent estrogenic (E) in character. Oral therapy was then instituted (fig. 1).

Case 05, M.G. was a colored, married, 30-year-old woman, para 2-1-1, who had had metrorrhagia for approximately one year. A curettage was performed December 10, 1938 to

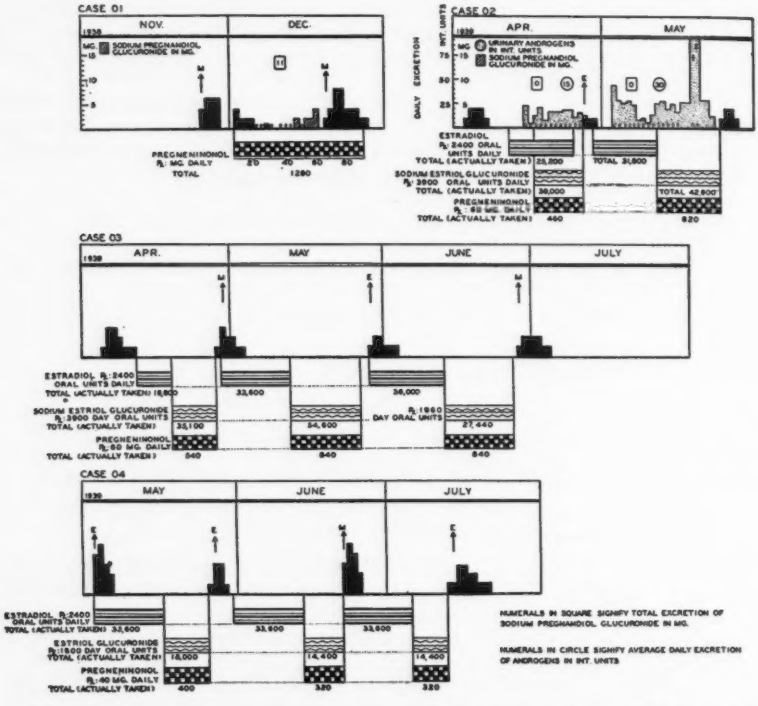

Fig. 1. Records of flowings, treatments, endometriotropic responses and of urinary titers of androgens and pregnandiol complex. *Cases* 01, 02, 03 and 04. Uterine flowing is indicated by solid black blocks. Biopsies are signified by ↑. *M* indicates mixed endometrium; *E* persistent estrogenic endometrium and *E*+ hyperestrogenic endometrium. Titers of androgens are indicated by stippling and those of the pregnandiol complex by the interlined blocks.

stop a depleting hemorrhage which had lasted 15 days and reduced hemoglobin and erythrocytes to 26% and 2,890,000 respectively. The endometrium was of estrogenic character. Oral therapy was begun immediately after the curettage (fig. 2).

Case 06, G.G. was a white, married, 24-year-old woman, para 0-0-0, who had had episodes of metrorrhagia for 2 years. A curettage was performed December 17, 1938 to check an episode of bleeding which had lasted approximately 2 months. The endometrium was of an estrogenic character. Oral therapy was begun immediately following curettage (fig. 2).

Case 07, A.B. was a colored, single, 17-year-old girl who had had menometrorrhagia for 3 years. A curettage was performed June 17, 1939 to stop a depleting uterine hemorrhage

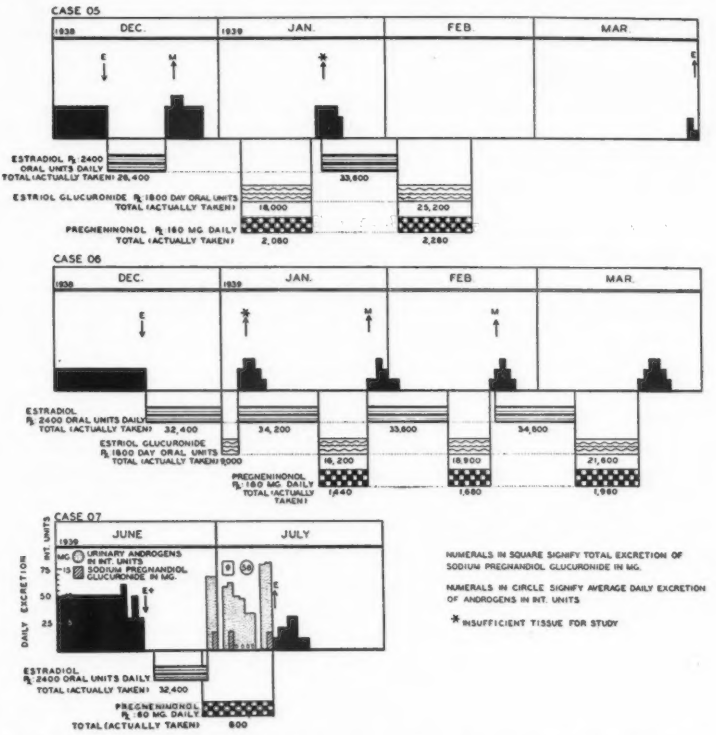

Fig. 2. RECORDS OF FLOWINGS, TREATMENTS, ENDOMETRIOTROPIC RESPONSES AND OF URINARY TITERS OF ANDROGENS AND PREGNANDIOL COMPLEX. *Cases* 05, 06 and 07. Explanations same as in figure 1.

which had lasted 6 months. The endometrium was of hyperestrogenic (E+) character. Oral therapy was begun immediately following curettage (fig. 2).

The endometriotropic responses of these patients to oral therapy are summarized in table 1.

DISCUSSION

All patients have shown good tolerance for pregneninonol. Doses as large as 160 mg. daily for 14 days (total dosage for a series, 2280 mg.) have been administered without untoward symptoms. No gastric disturbances were observed in any instance. Urinalyses indicated no signs of renal irritation. Many of these patients volunteered that they experienced a mild euphoria during therapy.

With one exception, *Case* 05, all patients bled cyclicly and in normal amounts and durations during active therapy. In the instance of the patient described as *Case* 05, bleeding following the second series of therapy was delayed approximately 1½ months, but was of normal duration and amount. A subsequent episode of bleeding was cyclic and quantitatively normal. Patients described as *Cases* 01 and 02 continued to have cyclic and quantitatively normal bleeding for several months after discontinuation of therapy. (The period of observation is short, as yet.) The patient described as *Case* 06, skipped a month of bleeding following discontinuation of therapy but subsequently had 4 cyclic and quantitatively normal episodes of flowing.

TABLE 1. SUMMARY OF ENDOMETRIOTROPIC RESPONSES

Case	Endometrium before therapy	Therapy	Endometrium during therapy
01	M	Pg (660)	M
		No R_x	MS
02	E+	Ed→Es+Pg (460)	E
		Ed→Es+Pg (820)	No tissue
03	E	Ed→Es+Pg (540)	M
		Ed→Es+Pg (840)	E
		Ed→Es+Pg (840)	M
04	E	Ed→Es+Pg (400)	E
		Ed→Es+Pg (320)	M
		Ed→Es+Pg (320)	E
05	E	Ed	M
		Es+Pg (2080)	Insf. tissue
		Ed→Es+Pg (2280)	E
06	E	Ed→Es	Insf. tissue
		Ed→Es+Pg (1440)	M
		Ed→Es+Pg (1680)	M
		Ed→Es+Pg (1960)	No tissue
07	E+	Ed→Pg (800)	E

Ed indicates estradiol; Es indicates estriol glucuronide; Pg indicates pregneninonol. Numerals in parentheses indicate total doses of pregneninonol.

In *Cases* 05 and 06, the irregularity of the patients might be explainable as due to the large doses of pregneninonol given (2280 mg. and 1960 mg. respectively). The effects of these larger doses should be investigated further. The effect of this oral therapy upon the cyclicity, duration and amount of uterine bleeding compares favorably, it seems, with that observed after injectional cyclic therapy (3). It is likely that this cyclicity may have been due solely to the estrogenic portion of therapy. This is being investigated in another similar series of patients.

Fourteen cycles of oral therapy, in which pregneninonol was used, are reported. The character of the endometria at the onset of episodes of bleeding was sampled by biopsies in 12 of these cycles. In 6 patients the endometria were of mixed progestational character, in 5 they were of estrogenic character and in 1 patient insufficient tissue for diagnosis was secured. Thus approximately 54% of the endometria during therapy showed some degree of progestational alteration. Only one of the 7 patients showed a mixed endometrium prior to therapy, an incidence of approximately 14%. It should be observed, however, that none of these endometria showed the marked and generalized progestational response characteristic of normal menstrual bleeding.

It is possible to compare these results directly with those obtained after injectional cyclic therapy with sex sterols. These latter data were reported in another communication by our group (9). Additional observations since that time are included in the data to be given. Injectional cyclic therapy (3), which employed progesterone in average doses for the series of approximately 35 to 40 mg., was given 32 patients. One hundred twenty-nine of these cycles of therapy were investigated as to endometriotropic responses by biopsies. Eighty-eight of the cycles terminated in bleeding from estrogenic endometria, 41 in bleeding from endometria which showed varying degrees of progestational alteration. Thirty-eight were mixed endometria and 3 normal menstrual endometria. The incidence, then, of some degree of progestational response during injectional therapy was approximately 33%. Since only 4 of the 32 patients showed mixed endometria prior to therapy, the incidence of endometria showing some degree of progestational alteration before therapy, was 12.5%.

The data indicate that pregneninonol when given orally together with estrogens

is apparently more effective in producing some degree of progestational alterations than progesterone when given by injection with or without estrogens.

The data secured in *Cases* 01, 02 and 07 regarding urinary titers of sodium pregnandiol glucuronide and of androgens indicate no significant alterations in these during the course of oral therapy. It may be inferred, then, that pregneninonol may exert specific progestational alterations in the endometrium without being altered into the pregnandiol-complex. The nature of the excretionary products derived from pregneninonol is not known.

Further and more detailed studies should be made to evaluate dosage and to investigate further endometriotropic responses. As yet, no normal progestational changes have been observed during therapy, although in one patient (*Case* 01) such a response was encountered at the next episode of bleeding which followed a cycle of no treatment.

SUMMARY

Oral therapy, embracing estradiol followed by estriol glucuronide and pregneninonol, was given cyclicly to 7 young women with functional meno-metrorrhagia. Observations during 14 cycles of this therapy are reported. Patients tolerated well the oral administration of as much as 160 mg. of pregneninonol daily for 14 days each month for several months. Bleeding of cyclic character and of normal amount occurred during treatment. The incidence of any degree of progestational response was approximately 14% in the endometria before therapy and approximately 54% during therapy. This therapy produced no significant alterations in the urinary titers of sodium pregnandiol glucuronide and of androgens of the patients investigated.

A part of the expenses incurred in these studies was defrayed by a grant from the Research Council of Duke University to one of us (E.C.H.).

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COMPARISON OF DIFFERENT PREPARATIONS OF AMORPHOUS AND ZINC CRYSTALLINE INSULIN

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IT IS CHARACTERISTIC of insulin that increases of dosage increase the duration more markedly than the intensity of the action. This rule holds true for both small (1) and large (2, 3) doses. There is therefore no fixed time limit for the action of any kind of insulin except as the increasing dosage finally reaches the point of fatal intoxication. Since the action of ordinary insulin may thus be extended as long as two days, it is reasonable to suppose that differences in the duration of action of different kinds of insulin will be correspondingly magnified. The standard method of testing the potency of insulin is with minimal doses. A number of authors have also compared the duration of action of different preparations of insulin using comparatively small doses (references in recent papers of Shepardson 4, Altshuler and Leiser 5, Meyers and Perkin 6, Wilder 7, Marble and Vartianen 9, Ricketts and Wilder 10). The literature is chiefly clinical and attempts at accurate comparisons in animals are strikingly few. Also, since differences which appear trivial or doubtful with small doses become clear and positive with large doses, a standard method for comparisons of this kind should logically be based upon large doses.

From several hundred experiments on this and related topics the following typical results may be selected for the present purpose. Two methods of testing have been studied, namely a convulsive method employing chiefly rats, and a method based on blood sugar curves.

CONVULSIVE METHOD

From trials in a large number of rabbits and a small number of mice, the following objections appeared in comparison with rats. (*a*) The strength is inadequate, inasmuch as too many animals die either from exhaustion or from acute hypoglycemia too suddenly to be saved by glucose. (*b*) Individual variations of tolerance and symptoms are too great. (*c*) There is difficulty in interpreting certain reactions. Particularly in certain individual mice, convulsions sometimes continue until death although the insulin dose is not large enough to produce such prolonged effects in most animals, and an autogenous hyperinsulinism or some other metabolic derangement must be suspected. More especially in rabbits there is sometimes a very prolonged weak state which is shown by blood sugar analyses to be due to hypoglycemia but which fails to cause convulsive attacks. All the above difficulties are to some extent encountered in rats, but on the whole the rat has proved in these experiments to be the least objectionable and the most reliable.

All ages and sizes and a number of different strains were tried. For preparation it appears to be practically immaterial whether the rats fast 12 or 24 hours or are taken directly from eating. The gastrointestinal contents or the glycogen reserves influence the interval elapsing before the first hypoglycemic convulsion, but do not particularly alter the duration of effect of large insulin doses. The insulin doses in this type of experiments have ranged from 20 to 200 U.

Several sites of insulin injections have been compared and within reasonable bounds no differences have been observed. In particular there has been no distinct difference between subcutaneous and intramuscular injections. Nevertheless the sites have been kept uniform and in a great majority of experiments the insulin has been injected in one hind leg. This has been done partly for comparison with amputation experiments but chiefly in order that the subsequent glucose injections might be given in other parts of the body. A mixture of injected glucose and insulin has thus been avoided, though no tests have been performed to prove whether such a mixture would make any difference.

As shown in former papers (2) the glucose required to relieve any hypoglycemic attack is merely the small amount necessary to raise the blood sugar and it bears no relation to the size of the insulin dose. Therefore the standard treatment employed

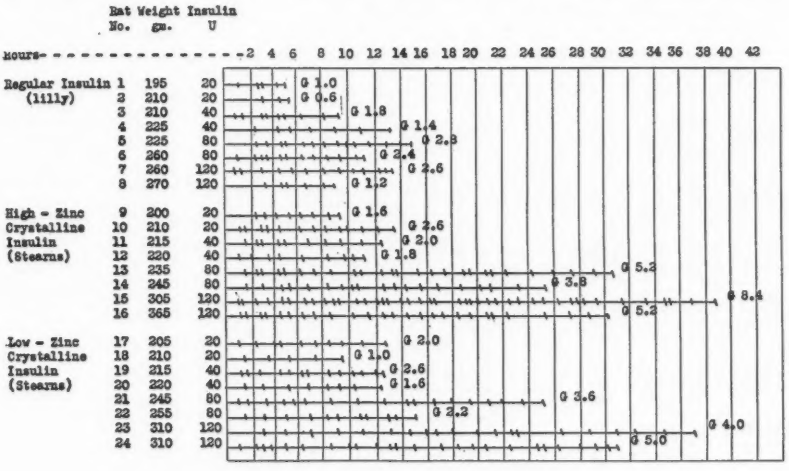

Fig. 1

for these attacks has been the subcutaneous injection of 0.2 gm. glucose (2 cc. of 10% glucose in Ringer solution). This is usually an effective antidote for an insulin dose ranging up into hundreds of units in a rat, whereas in a rabbit or cat receiving the identical insulin dose, 10 or 20 cc. of the glucose solution to raise the blood sugar is advisable. In an extremely severe attack a rat may need a second injection of 2 cc., which sometimes is given in the peritoneum. The well known convulsions are the usual manifestation of hypoglycemia, but sometimes quiet collapse and dyspnea may be still more dangerous and require treatment accordingly. In any case of doubt the hypoglycemic nature of the attack is verified by a blood sugar analysis in addition to the response to glucose.

In the two charts of such experiments the horizontal lines indicate the time, and each cross hatch represents a hypoglycemic convulsion or collapse treated with an injection of 0.2 gm. glucose. The figure with G at the end of the line gives the total grams of glucose used for antidoting the dose of insulin in each instance. The absence of any consistent ratio is evident as usual.

In figure 1, a comparison was made between the Lilly preparation of amorphous insulin marketed for clinical use, and two preparations of Stearns' solution of zinc

insulin crystals. One of these, designated as high zinc, contained 0.8 to 0.9 mg. of zinc per 1000 U, the same as the crystalline insulin used in my former experiments (2, 3). The other preparation, designated as low zinc, is the solution now marketed for clinical use, containing 0.2 to 0.3 mg. zinc per 1000 U.

It is obvious from figure 1 that no difference in the effect of crystalline insulin results from this degree of difference in zinc content. On the other hand there is a constant and striking difference between the duration of the effects of the crystalline and amorphous insulin, and this difference increases as the doses increase from 20 to 120 U.

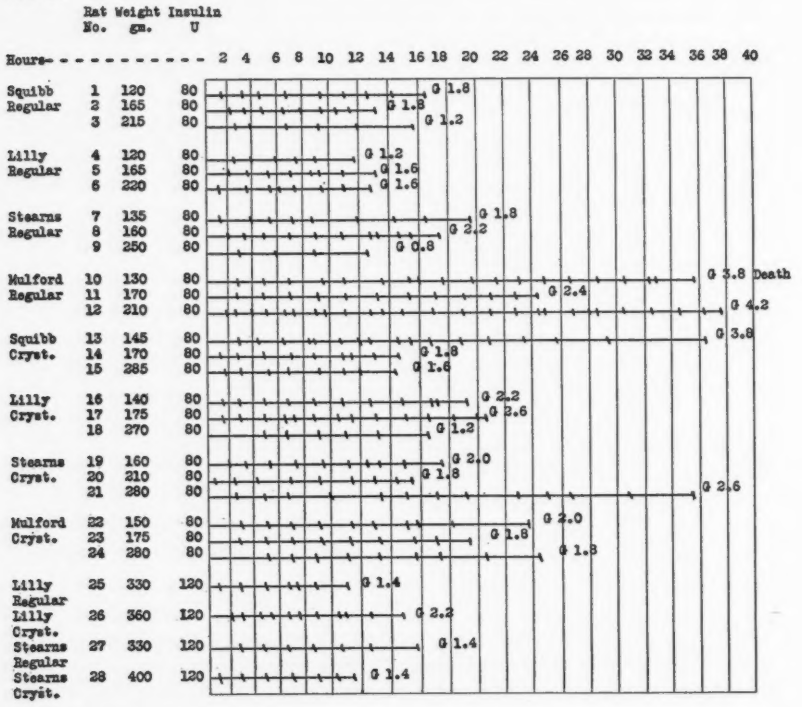

Fig. 2

Many series of experiments were performed comparing other insulin preparations, but owing to the limited supply, only a few with Connaught Laboratory insulin. The examples in figure 2 are selected to illustrate the typical findings. The insulin dose here was 80 U, but the results with doses from 60 to 160 U have been found similar. The following conclusions are derived from these observations.

The regular commercial preparations of Squibb and Lilly amorphous insulin are uniformly briefer in their effect than any of the other forms of insulin tested. The Stearns amorphous insulin has a slightly but definitely longer effect, individual exceptions being few and the general result being plain in any large series of rats. The prolonged action of the Mulford amorphous insulin is still more marked and positive. The few tests made with the Connaught Laboratory amorphous preparation indicate that it ranks among the longer acting insulins.

The effects of the zinc crystalline preparations regularly outlast those of the amorphous insulin of Lilly and Squibb as already stated. Some brief effects of crystalline insulin are intentionally illustrated, but the average differences are greater than here shown. On the other hand there is no general superiority of crystalline over amorphous insulin shown by the convulsive method, and the effects of the Mulford amorphous insulin in particular may outlast those of the crystalline preparations. Also no consistent differences between the crystalline insulin preparations of the different manufacturers are revealed by this method.

As usual, the insulin-glucose ratio has no reference to the potency of the insulin but is governed essentially by the duration of insulin action.

Different weights of rats are illustrated in the series with 80-unit doses, and 4 comparative examples with very large rats with 120-unit doses are appended. On

TABLE 1. BLOOD SUGAR VALUES OF A NORMAL DOG (FASTING) WEIGHT 12.6 KG.

Hours	Lilly Regular	Stearns Cryst.	Hours	Lilly Regular	Stearns Cryst.
0	129	132	0	110	90
	Insulin 5 U injected			Insulin 40 U injected	
1	70	89	1	82	66
2	62	78	2	78	44
3	68	86	3	70	49
4	74	90	4	62	48
5	61	98	5	55	40[1]
6	57	88	6	52	57
7	55	82	7	58	56[2]
8	69	89	8	57	93
9	89	84	9	115	63
10	96	88	10	117	67
11	98	86	11	130	105
12	109	93	12	119	114
13	120	99	13	112	150
14	102	105	14	122	140

[1] 20 cc. of 10% glucose solution injected subcutaneously, twice.
[2] 20 cc. of 10% glucose solution injected subcutaneously and 20 cc. intraperitoneally.

the whole there is an undoubted influence of body weight in shortening the insulin action and reducing the glucose requirement, thus altering the insulin-glucose ratio. This influence, however, is highly variable, sometimes lacking, and never in any mathematical parallelism with the difference in weight. Attempts to compare different series of rats on a per kg. basis of insulin dosage are therefore fallacious. Accurate comparisons can only be made with identical doses in a well matched series of rats.

Blood Sugar Curves

Two forms of experiments are possible: (*a*) one in which insulin is administered in the fasting state and the fall of blood sugar compared, the disadvantage being that the insulin doses thus used are small and the differences found are correspondingly small or doubtful; (*b*) one in which carbohydrate is administered either by mouth or as intravenous glucose, and the results are judged either by differences in the amount of carbohydrate required or by differences in the blood sugar curve with identical carbohydrate administration. Venous blood was analyzed for sugar by the Benedict picric acid method. The experiments presented are selected as typical from a large series, except that the small available supply of Connaught Laboratory insulin limited the tests with it. Two diabetic dogs were used; experiments on only one of them are shown but these are selected from numerous tests yielding the same type of results,

and it is believed that comparisons of many tests on one animal are more instructive for the present purpose than comparisons between different animals.

The experment with 5 U of insulin in a fasting normal dog (table 1) might be interpreted to signify that the Lilly regular insulin had a more prompt and powerful effect during the first 8 hours, but the effect of the Stearns crystalline insulin was more gradual and prolonged during a 13-hour period. The result with 40 U was more striking and positive. The Lilly amorphous insulin reduced the blood sugar to a hypoglycemic level, but the dog, though showing marked symptoms, had no convulsions or other signs of danger and was able to recover spontaneously. The Stearns crystalline insulin was both more prompt and more powerful in its action. The blood sugar fell faster and lower. Marked hypoglycemic symptoms were ignored until the 5th hour, when dangerous convulsions were controlled only by two subcutaneous injections, each of 20 cc. 10% glucose. The convulsion of the 7th hour seemed still more critical and 20 cc. was injected under the skin and 20 cc. into the peritoneum. Even

TABLE 2. BLOOD SUGAR VALUES OF A DIABETIC DOG (FASTING). WEIGHT 9.2 KG.

Hours	Lilly Regular	Stearns Cryst.	Hours	Lilly Regular	Stearns Cryst.
0	340	290	0	318	320
	Insulin 5 U injected			Insulin 40 U injected	
1	220	280	1	248	300
2	162	120	2	58	60
3	184	112	3	45[1]	65[1]
4	164	89	4	55	60
5	98	92	5	42[1]	63[1]
6	58	110	6	72	66[1]
7	78	135	7	48[1]	61
8	90	128	8	52	59[1]
9	140	142	9	75	55
10	255	280	10	68	60[1]
11	289	282	11	70	52
12	320	280	12	73	54
13	265	390	13	90	101
14	440	370	14	212	94
			15	326	86

[1] 20 cc. of 10% glucose solution injected subcutaneously.

after the giving of 8 gm. of glucose in this manner, the blood sugar remained much lower than with the Lilly amorphous insulin up to the 10th hour. The hyperglycemia at the 13th and 14th hours presumably represented a delayed effect of the injected glucose.

The dog used in the experiment reported in table 2 had been prepared by operation 10 months previously, when eight-ninths of the pancreas was removed. In addition to heavy carbohydrate feeding after the operation, the animal received 4 intraperitoneal injections of anterior pituitary extract (Young) of 2 to 5 cc. each. The writer has repeatedly called attention to the usefulness of partially depancreatized animals because of their freedom from indigestion and complications and the close imitation of clinical diabetes of any desired degree of severity. This particular dog was brought to such a stage of diabetes that glycosuria could be stopped by 3 days of fasting, but hyperglycemia persisted during 5 days, and glycosuria was then restored by the feeding of only 50 gm. of meat. A regular program of diet and insulin then arrested the condition and kept it stationary, as proved by repetitions of the fasting test during several months. The appetite and general condition were always excellent.

The experiment with 5 U of insulin showed a possible difference between the Stearns crystalline and the Lilly amorphous insulin, but it is within the limits of accidental variation. When 40 U was injected the blood sugar fell more rapidly with the amorphous than with the crystalline insulin and the symptoms at first were more violent. The reduction of sugar, however, was much more prolonged in the case of the crystalline insulin, with a double result: (*a*) a total of 5 glucose injections was required instead of 3; (*b*) in spite of this the blood sugar was much lower after 14 or 15 hours than in the case of the amorphous insulin, which had then lost all its effect so that the hyperglycemia was rising to a maximum.

The same diabetic dog (table 3) was given 40 U of insulin, followed in one hour by the feeding of 150 gm. meat. Two preparations of amorphous and two of crystal-

TABLE 3. BLOOD SUGAR VALUES IN A DIABETIC DOG. WEIGHT 9.2 KG.

Hours	Lilly Regular	Lilly Cryst.	Stearns Cryst.	Connaught Laboratories
0	318	360 Insulin 40 U injected	320	360
1	—	286 Fed 150 gm meat	300	260
2	58	58	60	86
3	45[1]	46[1]	65[1]	54[1]
4	55	36	60	57
5	69[1]	58[1]	63[1]	36[1]
6	72	63	66[1]	51
7	48[1]	46[1]	61[1]	56[1]
8	52	71	55	66
9	75	60[1]	60[1]	57
11	68	67	52	69
11	70	72	54	92
12	73	99	101	102
13	90	125	94	108
15	212	190	86	196

[1] 20 cc. of 10% glucose solution injected subcutaneously.

line insulin were thus compared. During the first 5 hours there was no positive difference in blood sugar, symptoms or treatment. At the 6th hour with the Stearns crystalline insulin convulsions demanded an extra glucose injection. The analytical results, completed later, showed actually lower blood sugars in tests with the other kinds of insulin, but the symptoms were not threatening as with the Stearns crystalline insulin. At the 7th hour glucose injections were needed with all varieties of insulin. At the 9th hour the symptoms demanded glucose only in the case of the two crystalline insulin preparations. The blood sugar was even lower with the Connaught Laboratory amorphous insulin, but the symptoms were slight and recovery occurred unaided. The net results were as follows: (*a*) each of the amorphous insulin preparations required 3 glucose injections, the Lilly crystalline required 4, and the Stearns crystalline required 5; (*b*) nevertheless the blood sugar at the 13th and 15th hours was lowest with the Stearns crystalline insulin. The advantage of the Lilly crystalline insulin over the Lilly and Connaught amorphous preparations was evident chiefly through the extra glucose injections.

The same diabetic dog (table 4) was given 20-unit doses of different insulin preparations, and 2 hours later was fed 150 gm. meat. The periods of blood sugars below 60 mg.% are marked off by horizontal lines. Hypoglycemic symptoms were very marked during these periods, but the meat sufficed for protection and no glucose administration was required. The following results are seen in the table. (*a*) There was

no general superiority of amorphous over crystalline insulin in speed of action. Also, the exceptionally early hypoglycemia from Squibb crystalline insulin in this instance does not establish a rule. On the whole the beginning of the hypoglycemic effect may be considered as nearly equal with all the insulin preparations except protamine zinc insulin. (*b*) The characteristically brief action of the regular amorphous insulin of Lilly and Squibb is confirmed by the fact that these were the only ones permitting blood sugar values above 200 mg. at the 11th hour, while the values with all the other insulin preparations were much lower at this hour. (*c*) There were marked differences between the amorphous insulin preparations of different manufacturers, the particularly prolonged effect of the Mulford preparation being confirmed. (*d*) Differences between the different brands of zinc crystalline insulin were also illustrated, the Stearns preparation having a longer effect than any of the others. (*e*). On the whole the crystalline preparations had a more prolonged effect than the amorphous; the only exception was found in the Mulford products. The crystalline preparations

TABLE 4. BLOOD SUGAR VALUES IN A DIABETIC DOG. TESTS WITH VARIOUS FORMS OF INSULIN

Hours	Lilly Reg.	Lilly Cryst.	Stearns Reg.	Stearns Cryst.	Squibb Reg.	Squibb Cryst.	Mulford Reg.	Mulford Cryst.	Sahyun HCL	Protamine Zinc
0	348	336	324	380	336	336	348	364	376	436
					Insulin 20 u injected					
1	340	302	320	360	242	103	248	260	400	360
2	140	114	208	282	97	50	98	87	120	360
					Fed 150 gm. meat					
3	80	46	124	101	39	39	49	37	98	320
5	49	40	72	51	34	45	41	34	50	284
6	53	39	40	45	41	35	40	51	58	260
7	37	29	50	51	42	—	43	59	46	220
9	65	51	53	53	80	50	46	89	36	210
11	208	77	88	50	202	120	76	109	80	188
13	—	200	144	85	246	240	109	272	170	164
15	320	246	300	132	440	300	176	364	260	67
17	308	320	324	232	440	334	244	272	270	41
22	364	356	374	376	480	364	336	364	344	42

of Lilly and Squibb had more enduring effects than their amorphous insulin. The longest acting crystalline insulin, namely that of Stearns, surpassed in this respect the longest acting amorphous insulin, that of Mulford. (*f*) Included in the series was a test of Sahyun's insulin hydrochloride (8) an amorphous highly purified product characterized especially by the absence of zinc. It is thus evident that zinc is not an essential component for obtaining a thoroughly typical insulin curve of blood sugar. (*g*) Protamine zinc insulin is obviously in a class apart from all the other insulin preparations. The beginning of symptoms with a blood sugar level of 67 mg. was delayed until the 15th hour, and the hypoglycemia continued through a long subsequent period not shown here, during which glucose injections also were required. (*h*) The differences between the insulin preparations, apart from protamine, were accompanied by marked differences in clinical behavior for periods of 2 or 3 hours. It must again be noticed that both the absolute effects and the differences can be increased with increased dosage.

In a normal young man (table 5), an injection of 5 u in the fasting state revealed no distinct difference between Lilly amorphous and Stearns crystalline insulin. An increase of the dose to 20 u resulted in hypoglycemic symptoms which demanded the

TABLE 5. BLOOD SUGAR VALUES IN A NORMAL MALE, (A.C., AGE 21 YR., WEIGHT 70 KG.)

Hours	Lilly Regular	Stearns Cryst.	Lilly Regular	Stearns Cryst.
0	127	136	115	102
	Insulin 5 U injected		Insulin 20 U injected	
1	92	125	110[1]	70[1]
2	93	86	87	75[1]
3	90	91	91[1]	74[1]
4	97	117	77[1]	77[1]
5	91	111	79	75[1]
6	93	109	85	79
7	96	102	79	88[1]
8	90	98	92	107

[1] Drank 50 cc. of 20% glucose solution with 2 cc. lemon juice.

drinking of 50 cc. of 20% glucose solution 3 times during a 4-hour period in the case of the amorphous insulin, and 6 times during a 7-hour period in the case of the crystalline insulin. In this case the action of the crystalline insulin was more prompt, in addition to being longer continued.

In a normal young woman (table 6), comparisons of two amorphous and two crystalline preparations yielded results which were slightly but distinctly longer for the crystalline products. Peculiarly, the increase of the dose to 15 U failed to accentuate the result. A further increase was made to 30 U, and glucose was given orally at the 3rd hour in each instance. Here the effect of the Lilly amorphous insulin

TABLE 6. BLOOD SUGAR VALUES IN A NORMAL FEMALE, (I.K., AGE 22 YR., WEIGHT 65 KG.)

Hours	Lilly Regular	Lilly Cryst.	Stearns Regular	Stearns Cryst.
0	125	132	111	119
		Insulin 5 U injected		
1	109	123	98	100
2	118	111	97	113
3	100	112	92	107
4	106	111	99	93
5	104	109	106	96
6	98	98	109	92
7	100	95	102	93
0	142	125	136	129
		Insulin 15 U injected		
1	103	77	82	98
2	86	76	55	75
3	87	79	73	60
4	91	88	74	61
5	88	83	72	59
6	92	82	61	79
7	97	84	78	76
0	136			127
		Insulin 30 U injected		
1	88			75
2	65			85
3	58[1]			75[1]
4	65			75
5	60			75
6	62			45
7	73			50

[1] Drank 50 cc. of 20% glucose solution with 2 cc. lemon juice.

was the more rapid, and that of the Stearns crystalline insulin was the more prolonged. This difference was greater than might be gathered from the table, since with the amorphous insulin the subject was well and comfortable from the 6th to the 7th hour and recovered spontaneously, while with the crystalline insulin she was practically unconscious during this time and required considerable carbohydrate for recovery afterward.

In a male diabetic (table 7), the comparison of 5-unit doses showed slight if any difference between Lilly amorphous and Stearns crystalline insulin as respects influence upon the fasting blood sugar curve. Comparison of 20-unit doses showed no distinct difference in the first 4 hours, but at the 5th and 6th hours glucose administration was required in the case of the crystalline insulin. In this instance the effect of the crystalline insulin was approximately as prompt and in the later hours was more powerful than the effect of the amorphous insulin.

TABLE 7. BLOOD SUGAR VALUES IN A MALE DIABETIC PATIENT, (*A.N.*, AGE 41 YR., WEIGHT 55 KG.)

Hours	Lilly Regular	Stearns Cryst.	Lilly Regular	Stearns Cryst.
0	234	230	242	223
	Insulin 5 U injected		Insulin 20 U injected	
1	206	211	239	214
2	171	199	166	187
3	159	166	150	159
4	156	150	100	87
5	142	150	80	60[1]
6	156	142	83	87[1]
7	171	148	79	87

[1] Drank 50 cc. of 20% glucose solution with 2 cc. lemon juice.

In a young female diabetic (table 8), there was a distinctly more prolonged effect upon the fasting blood sugar with 5 U of Stearns crystalline insulin than with Lilly amorphous insulin. With a dose of 20 U, the crystalline insulin demanded administration of glucose orally 5 times as compared with 4 times for the amorphous insulin.

TABLE 8. BLOOD SUGAR VALUES IN A FEMALE DIABETIC PATIENT, (*R.K.*, AGE 25 YR., WEIGHT 45 KG.)

Hours	Lilly Regular	Stearns Cryst.	Lilly Regular	Stearns Cryst.
0	357	312	300	319
	Insulin 5 U injected		Insulin 20 U injected	
1	272	288	272	278
2	230	189	214	195
3	182	150	136[1]	150[1]
4	175	127	93[1]	77[1]
5	164	113	93[1]	105[1]
6	182	131	83[1]	91[2]
7	187	140	136	123
8	195	154	91	93

[1] Drank 50 cc. of 20% glucose solution with 2 cc. lemon juice.
[2] Drank 50 cc. of 20% glucose solution with 2 cc. lemon juice, twice.

CONCLUSIONS

The duration of effect of any kind of insulin, and the differences between different kinds, are not absolute but are proportionate to the size of the dose. Testing for duration therefore differs from the usual method of testing potency, in that the clearest results require large rather than small doses.

A convulsive method, especially in rats, establishes the identity of action of crystalline insulin with a content of 2 or 3 to 8 or 9 mg. of zinc per 1000 U, but a decidedly longer time of action of this crystalline insulin than of Lilly or Squibb amorphous insulin. Differences between different brands of amorphous insulin, heretofore supposed to be identical, are also clearly revealed by this method. On the other hand no consistent differences are shown between different brands of crystalline insulin.

The method of blood sugar curves in normal and diabetic animals and patients is more delicate, in that it confirms the above-mentioned differences and also reveals others. Different duration of action is thus demonstrated for the amorphous and crystalline insulin of the same and different manufacturers. The crystalline insulin preparations as a group have a more prolonged action than the amorphous insulin preparations. The longest acting amorphous insulin, namely that of Mulford, has a slightly shorter effect than the longest acting crystalline insulin, namely that of Stearns.

The prolonged action of crystalline zinc insulin appears sometimes to be a retarded action, especially with small doses, but more commonly and typically the fall of blood sugar is as rapid and early as with amorphous insulin. The prolonged action thus results in a greater total effect, one indication of which is the larger quantity of glucose usually required for antidoting a given dose of crystalline as compared with amorphous insulin.

The total time of action and the differences between the insulin preparations mentioned are not comparable with the retarded action of protamine zinc insulin, which is characterized by a long delay in the beginning of its effect in addition to the extremely long continuance. Nevertheless the differences between the other insulin preparations are important enough to deserve attention both theoretically and practically.

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THREE CASES OF MASKED HYPOTHYROIDISM HAVING ABDOMINAL SYMPTOMS

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Conditions due to absence or marked diminution of thyroid function are well known and diagnosis is comparatively easy. There are mild degrees of the condition in which the glaring symptomatology of cretinism or myxedema in the adult is not conspicuous. In the presence of a past history of thyroidectomy, one's attention may be directed to the possibility of a post-operative hypothyroidism, but in the absence of such in an individual who had been reasonably well, hypothyroid states too often escape pointed attention. Masked hypothryoidism may be the underlying cause of serious gastro-intestinal disturbances and may imitate symptoms of chronic involvement of abdominal structures. Hertoghe, who first recognized masked hypothyroidism, drew attention to the part played by consanguinity and heredity. It may be assumed that different organs in the body have different thresholds for thyroxine, that some require more and some require less than other organs for normal physiological functions. This is suggested in the digestive tract and cardiac apparatus.

In this condition there appears to be no regularity of symptoms as can be observed in hyperthyroidism. Instead the symptoms are fleeting and present in varying degrees and combinations at different times. One's suspicions might be aroused in the chronically fatigued type with mental sluggishness, delayed menstruation, modified genital organ development, pallor and scanty hair. Modifications of the classic symptoms seen in distinct myxedema appear in most of the cases. They are not striking and often escape detection. The gastrointestinal symptoms as arrayed by Ramsey are, anorexia, flatulence (especially after meals), constipation, occasional nausea and rare vomiting, gastric hypoacidity and achlorhydria, and abdominal pains which may simulate gastro-duodenal ulcer, cholecystitis and appendicitis. The symptoms more helpful in the diagnosis of hypothyroid states are pains in the abdomen, pallor and dry skin, blood pressure never above normal and usually below, tendency to subnormal temperature, mental depressions and poor memory, sparseness or absence of pubic hair and retarded developments of the genitalia, low B.M.R. and leucopenia with generally a high relative lymphocyte count. In order to make the cases reported below clearer all irrelevant findings are omitted.

1. *Case 1.* A woman, 22 years old, developed over a space of several months a lack of interest in her work and occasionally was nauseated in the mornings. She complained of pains in the lower right abdomen, was diagnosed as having appendicitis and operated upon. The pains persisted after the appendectomy.

The physical examination gave normal findings except for a blood pressure of 90/60; the skin felt dry, the pulse was 60, she was about 20 pounds overweight, and was tender to pressure up and down the right side of the abdomen.

The laboratory and x-ray findings were normal with the exception of the white blood cell count of 2,700, lymphocytes of 72%, a 25% 6-hour gastric stasis and a mobile cecum, and a B.M.R. of −21%.

She was placed on thyroid extract, beginning with 1 grain a day and increased a grain

each day until 12 grains a day was taken. One month later she was perfectly well, had lost 12 lb., the white cell count was normal, the blood pressure 118/86 with the B.M.R. still −21%.

Case 2. A married woman, 31 years old, came under observation for chronic constipation. She gave a history of having had many headaches, mostly occipital and said she had a 'slow heart.' She began menstruating at 17, had always been somewhat irregular and had been pregnant 3 times, miscarrying in each instance. She had had considerable gynecological attention and had even been judged as syphilitic although results of several Wassermann and Kahn tests had always been negative. At times she had nausea and vertigo which were attributed to the constipation. She had been fatigued for years, the last year more noticeably. She had attacks of precordial pain, was nervous, had insomnia and mental depressions and stated that she occasionally had swellings of her eyelids and hands "which was part of an eczema." For this she had had treatment for allergy over several years without much benefit. She stated also that she was "inclined to anemia" and had courses of "liver and iron injections."

On physical examination she had a blood pressure of 95/82. She looked pale but her skin was moist. She had very scanty pubic hair. Her pulse rate was 56, temperature (rectal) 97.4°. There was a general tenderness to pressure all over the abdomen, more particularly in both inguinal regions. Several small hemorrhoids were present. There was a quick response but slow relaxing Achilles' tendon reflex, a slight patella reflex, and no superficial abdominal reflexes.

Urine contained a trace of albumin and was high in indican. The blood showed hemoglobin (Sahli) 79%, red blood cells 4,100,000, white blood cells 5,100 with 62% lymphocytes, 33% polymorphonuclear leucocytes, 4% mononuclear leucocytes, 1% eosinophiles. Other than an ileo-colonic stasis, the x-ray examination showed nothing abnormal. The B.M.R. was 8%.

This patient was placed on ascending doses of thyroid extract as in the former case. When she was taking 6 grains a day without dietary assistance, the bowels moved normally for the first time in years. This, however, did not continue in daily movements although the constipation was much improved, but with the increase of fresh fruits, amounts of fluid and granulated agar-agar at breakfast, daily bowel movements were established. In the fifth week from the beginning of taking thyroid she menstruated more satisfactorily than for some years and in a 10-month observation period this has continued at about a 30-day cycle and continues to be normal. Fatigue, skin phenomena and abdominal pain disappeared in about 1 month; the blood picture changed to 7,200 white blood cells with a lymphocyte count of 43% and a corresponding increase of the polynuclear leucocytes. The B.M.R. continued to be −4 and −10.

Case 3. A single woman of 27, who worked as a saleswoman, suffered abdominal pains which affected her intermittently for 7 months and later became more pronounced and steady. Among the complaints enumerated were constant cold hands and feet, attacks of cardiac palpitation, sleepiness during the day, loss of appetite, flatulency after meals with a pressing feeling in the stomach region, oligomenorrhea, ear noises, dry mouth at times and constipation. At times she had pain in the upper right flank and had been diagnosed as having a "diseased gall-bladder." She stated that she had always been about normal in weight but in the last 15 months had gained 19 pounds for no reason that she could assign.

On examination it was found that she had several deciduous teeth, her breath was foul and the heart sounds were muffled with occasional extra systoles. There was a vague sense of tenderness up and down the right abdomen. All her reflexes were brisk. She had an old anal fissure but had practically no local distress from it. The blood pressure was 112/90. Other than for a moderate degree of stasis in the colon the results of a roentgenographic examination were normal. The blood picture was also not significant in that the red cells were 4,100,000, hemoblogin 82% and white cells 6,200 with lymphocytes 42% and polymorphoneuclear leucocytes 64%. The B.M.R. was −10%.

She was placed on an ample diet and given injections of glycerophosphates daily for 2 weeks with no benefit. On the third office visit she casually stated that she had always

perspired freely but that in the last year her skin was dry. This, the history and a low B.M.R., suggested the possibility of a low thyroid condition and she was placed on 3 grains of thyroid extract a day, the diet being continued. Within a week she claimed to be feeling "immensely better." The thyroid extract was continued for 2 weeks and then reduced to 3 one-grain tablets a day. On the fourth visit, a month later, she claimed to be feeling well, her appetite was good, fatigue and indigestion were gone, the dryness of mouth and skin had disappeared and she had lost 8 pounds in weight. The extra systoles had disappeared, her blood pressure remained about the same, hemoglobin was 90%, the white blood cells were 6,700, the blood count remaining about the same. Her bowels were moving daily. The B.M.R. was −6.

DISCUSSION

There seems to be an occasional case of masked hypothyroidism which presents itself with complaints of abdominal pain. In these cases suggestions of a partial type of myxedema may be deducted from the history and physical findings. They present low blood pressure, leukopenia and low basal metabolic rate, all of which can be determined by laboratory procedure. These cases, because they come under observation with abdominal complaints, may escape proper diagnosis and are dramatically benefited by thyroid therapy. In them there seems to be a diversity of complaints and physical findings. The significance of the low thyroid condition is liable to be lost despite the fact that it is the main cause of the illness. If the possibility of the condition is kept in mind, one may be able to read into the history and physical findings a suggestion of the condition although usually it is not suspected until the low blood pressure, blood findings and low basal metabolic rate leads one to the proper diagnosis. Why there is abdominal pain and tenderness in this condition is not understood. It may be neurogenic in origin; it certainly is not due to colonic stasis or constipation.

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THE MEDICAL TREATMENT OF HYPERTHYROIDISM

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THE CONCEPTION that treatment of Graves' disease (1) is primarily surgical, is widespread despite the fact that American as well as European literature contains numerous reports of satisfactory results with non-surgical treatment in selected cases. It is undoubtedly true that since Plummer and Boothby (2, 3, 4) inaugurated routine preoperative iodine preparation, the prognosis of surgical procedures in Graves' disease has been markedly improved. It is also true that a high percentage of cures is obtained by subtotal thyroidectomy, but the results are by no means universal. Because of these operative failures, and because of those cases not amenable to surgery, it is the immediate concern of the internist, from a practical point of view, to continue his search for a satisfactory medical treatment.

The mode of action of different types of therapy is still far too speculative to be discussed in this purely clinical paper, but one must admit that it is extremely difficult for patho-physiologists to comprehend how a large number of hyperthyroids are cured or helped by simply cutting out a piece of an overactive gland. It appears likely that the enlargement of the thyroid is by no means the cause of the hyperthyroidism, but rather a sequel. The relationship between the size of this gland and the degree of hyperthyroidism has never been determined. Even the exact histological study of Sunder-Plassmann (5), in which he was able to show much damage to the local sympathetic fibers as a result of subtotal thyroidectomy, by no means covers the entire subject, as was first pointed out by Bauer (6, 7), and his conclusions were too far reaching. This lack of information as to the theoretical considerations relating to the mechanism of action confronts the research worker in the field with an important problem directly related to treatment.

In the face of present day literature, it is impossible for us to agree with Richter (8), when he claims that the treatment of Graves' disease is entirely surgical. On the other hand, we cannot concur with Jenkinsons' view (9) that every case should be treated primarily medically and only failures then subjected to surgery. We hold with the majority of workers that a certain group of cases are surgical, the remainder medical, to be treated surgically only after non-operative failure. Most workers agree, that as soon as it becomes apparent that the results of medical therapy are not good, surgery should be immediately resorted to with the least possible delay so that the optimal time for operation is not missed. On the other hand, too early surgical intevention, especially in early acute Graves' disease, is also not advisable because postoperative crises are most common in these cases, even with preoperative iodinization, and incomplete results as well as recurrences are relatively frequent. To this group belong the cases with diffuse hyperplasia, as pointed out by Barr (10). In contrast, patients whose hyperthyroidism is partly burned out by medical treatment, respond later to surgery with much better results.

Redisch (11, 12) considers the surgical indications as follows (Prague 1934).

(*a*) Those cases in which a local mechanical disturbance exists in conjunction with the hyperthyroidism even when this mechanical disturbance is mild.

(*b*) Cases of hyperthyroidism in which one or more nodules can be felt, firmer than the remaining gland, most commonly found in older goiters with secondary toxicity.

(*c*) Failures of conservative therapy, again stressing the point that one must not wait too long.

(*d*) Wherever an immediate danger of circulatory involvement is imminent (especially the development of auricular fibrillation in the absence of cardiac failure).

Harris and Rose (13, 14) express a similar point of view (1936) but favor x-ray over medical treatment because of the economic aspects, an argument which could of course likewise be presented by the surgeon.

Means and his associates (15) have worked out an excellent scheme to prognosticate the operative risks, so that their cases come to surgery not only satisfactorily prepared, but also very well selected. They have had a postoperative mortality of only 2.6% since the advent of routine preliminary iodinization. It is of great interest to note that in Means' clinic at the Massachusetts General Hospital in the period from 1923 to 1935, 97.6% of all cases were operated on, 1.5% treated with iodine alone, and 0.3% with x-ray and surgery, whereas since 1935 (personal communication) the percentage of medically treated cases (iodine alone) has risen to from 12 to 15%. In Biedl's clinic (Prague, 16) almost the same postoperative mortality figures are found, but there only 30% of the hyperthyroids were operated on. Of the remaining 70%, 10% were treated with iodine alone, 60% by x-ray alone, and 30% by x-ray and iodine with a mortality figure much under 1%. In this group are not included those cases of very mild hyperthyroidism with B.M.R. of less than +15, all of whom were treated conservatively and not considered for surgery (W.R.).

The Various Types of Conservative Therapy

Means and his associates (15) point out in their reports that of all the conservative methods available in the treatment of hyperthyroidism, only two are worthy of consideration at the present time as being complete in themselves, namely iodine and x-ray. Redisch (10) published a survey on the therapy of hyperthyroidism in 1934 and came to essentially the same conclusions.

There is, however, a third possibility which may show promise in the future: the treatment with normal blood serum, utilizing the antithyroid principle of Blum (17) which he called 'katechin.' It cannot be denied that in mild cases some favorable effects are often seen (18–21). One can certainly not recommend the employment of this means as a general rule at the present time because the nature of the material and its mode of action are entirely unknown.

It is postulated (17) that this inhibitory substance is produced physiologically in the body, circulates in the blood stream, and aids in the regulation of available thyroxin to the tissues. Certainly the theory of antibody production is in complete disagreement with every hormonal concept up to the present time and it may have been just such substances as this antithyroid principle with which L. Loev and others have been dealing (22–26). Very similar to 'katechin,' as noted by Means, is the 'antithyroidin' of Moebius (27), who was able to use effectively the serum of thyroidectomized sheep in hyperthyroidism. Here apparently, the antithyroid principle is unopposed by the thyroid hormone, accumulates in the blood stream and so is free to lower the recipient's own thyroxin activity. Moebius also employed a substance called 'rhodagen,' obtained from the milk of thyroidectomized goats (Burghart and Blumenthal). Successful reports continue to appear in the literature from time to time.

It seems, furthermore, from a clinical point of view, that patients who have

been previously iodinized are more susceptible to the inhibitory action of normal blood serum. Whether this depends on the degree of iodine saturation of the thyroid hormone, or simply on the level of iodine in the patient's blood serum, is not known. The whole problem is wide open for investigation at the present time and the writers are engaged in an attempt to elucidate this antithyroid principle.

There are a few remaining methods which may occasionally be employed as supplements, especially in mild cases. Some of these are purely symptomatic as the sedatives, of which the bromides require particular attention. Bromine replaces iodine even in thyroid and so disturbs the iodine balance (28–31) still further, adding to the therapeutic difficulties. Other types of treatment act on the vegetative nervous system, the relationship of which was pointed out particularly by Bramm (32), and and others (33). Hospitalization itself, is an important factor, particularly when high carbohydrate, high vitamin, low protein diet is used (16, 34). Marine and his coworkers (35) have done most of the pertinent experimental investigation on the vitamins but clinically, different ones have been stressed by various workers (15, 36, 37). Gynergen, which was employed with injurious effect in the past, has been universally abandoned.

It has been noted in Central Europe that even severe cases of hyperthyroidism sent to certain mountain climates (about 1000 to 1200 meters above sea level) showed an entirely unexplained but definite improvement (38, 39, 40). The large number of experiments designed to improve hyperthyroidism by acting on the correlation mechanism of the endocrine organs (41, 42, 43) need not be considered here because we are concerning ourselves only with nonsurgical methods which act directly on the thyroid. By the same token the complications of organic symptoms are not discussed.

Treatment with Iodine Alone

The relationship between iodine and the thyroid gland, particularly as regards function, was recognized as early as the beginning of the last century by Straub (1819) (44), and Coindet (1820) (45). After the syndrome of Flajani (46) was classically described by Graves (1) and von Basedow (47), iodine became the basis for treatment, with variable degrees of success (48), until that time when Kocher first described his hyperthyroidism presumably caused by iodine (49). This conception of the great Swiss surgeon caused such a sensation that the use of iodine was almost completely abandoned. Today, however, we are sceptical of the validity of Kocher's conclusions (15, 50).

In 1917 iodine was reintroduced into the therapy of hyperthyroidism by Chvostek (51). He reported improvement in some of his cases but aggravation of the condition in others. Similar results were obtained by Neisser (52) in 1920 and by Loewy and Zondek (53) in 1921. In the same year Plummer and Boothby began their extensive investigation which led to the universal acceptance of Lugol's solution in preparation for surgery. Starr and Means (54), Mason (55), and Read (56) gave added impetus to the renewed use of iodine, and with this came the desire on the part of many workers to find some classification which would serve as a general basis for indicating iodine treatment (51–65).

Plummer and the other workers at the Mayo clinic reaffirmed the experience that iodine causes a great improvement in some patients, has no effect in others, and makes still others clinically worse, and on this basis differentiated between 'exophthalmic goiter' and 'toxic goiter.' Today there is no doubt that this differentiation cannot be made, particularly because exophthalmos is an extremely variable and inconstant sign in hyperthyroidism with no definite relation to the action of iodine. Biedl and Redisch will not admit to inherent symptomatic nor marked histologic differences between the real Graves' disease on the one hand, and hyperthyroidism on the basis

of an old goiter on the other, but feel rather that the hyperfunction of the thyroid gland has very different manifestations according to the constitutional state of the person affected. Real Graves' disease involves individuals with the particular habitus of the type variously called 'status thymico-lymphaticus,' 'asthenic,' 'hypoplastic,' and these workers point to the changes found in the blood picture of Graves' disease after iodinization for confirmation.

Hertz and Lerman (66) found the same changes in the blood picture independently. Despite all classifications, the therapeutic test with iodine remains as yet the best method of ascertaining a patient's response, but even this should not be attempted with old goiters become toxic.

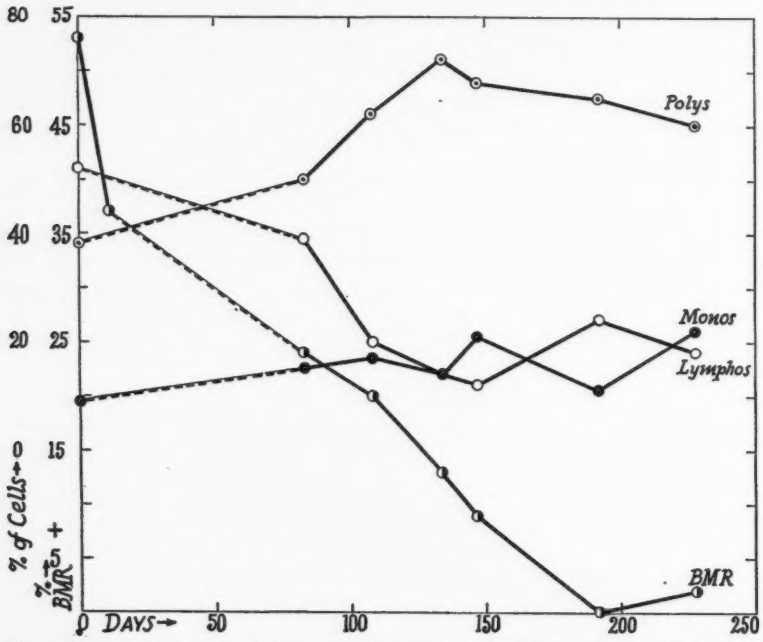

Fig. 1. The patient is a 17-year-old white girl on whom a thyroidectomy had been performed 2 years before admission. Her complaints started 1 year after the surgery was completed.

The official Lugol solution in America contains 5 gm. of iodine and 10 gm. of potassium iodide in 100 gm. of water, giving 126 mg. of iodine in each 1 cc. of solution. According to Boothby one begins treatment with 78 mg. iodine daily (0.6 cc.) and works up to 100 to 300 mg. Because of the fact that sodium is known to cause no damage to the heart and circulation as does potassium, Biedl and Redisch employed a solution containing pure iodine 0.1 gm., sodium iodide 1.0 gm., and water 10 cc., making 70 mg. of iodine in 15 drops of solution, which was the average daily dose. Meanwhile, it was shown by Keller (67), in his work on the electric charge of molecules, that whereas in vitro potassium and sodium behave similarly, in the organism this is by no means true, which may explain the better results with sodium. The above mentioned solution (sodium iodide) was given for periods of 8 to 12 days followed by rest periods of 3 to 5 days. This procedure seems to avoid to a great extent the negative phase of continued iodine administration.

In Biedl's clinic (68) about 10% of the cases with favorable results were completely and permanently cured, 40% entirely symptom free so long as iodine was administered, and 50% almost symptom free but still showing some manifestations of the condition. The 'toxic symptoms' of acute Graves' disease (diarrhea, restlessness, insomnia) reacted especially favorably to iodine. The fact that the percentage of cases treated by iodine alone in Means' clinic has increased remarkably during the last 3 years, shows that the pendulum may be once again swinging towards the medical treatment of hyperthyroidism in this country.

The prophylaxis of goiter and its distribution are problems in themselves and we will not discuss them here except to point out the following.

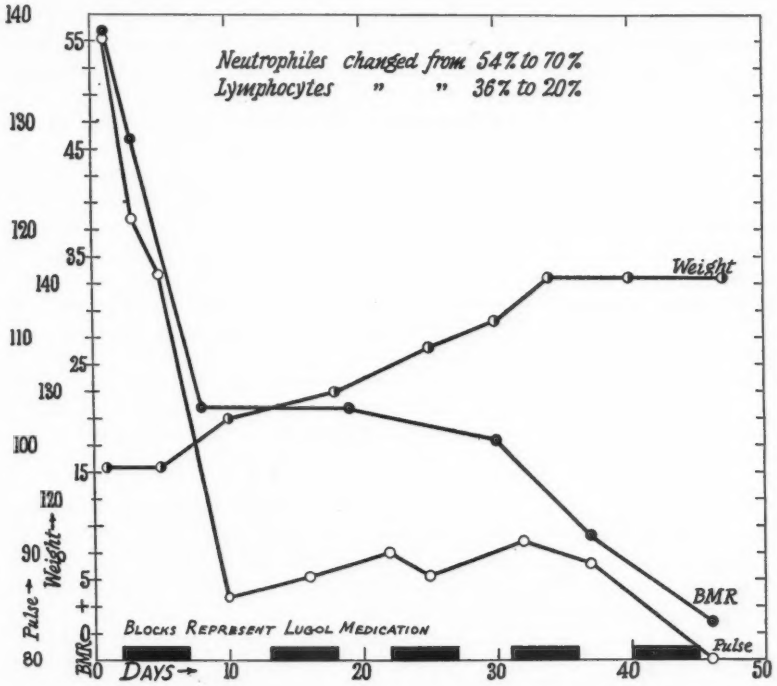

Fig. 2. The patient is a 24-year-old white woman who had a thyroidectomy 8 months before this study.

(*a*) Patients with real Grave's disease do not belong to that group particularly susceptible to iodine poisoning, De Quervain (69, 70, 71) to the contrary.

(*b*) There need be no particular fear of iodine poisoning in pubertal goiter.

(*c*) Toxicity symptoms after iodine occur relatively frequently in *a*), goiter with pregnancy, *b*), goiter with lactation, and *c*), individuals over 30 years of age with old nodular goiter.

X-Ray Treatment

Judging by the amount and quality of American contributions to the literature on the treatment of hyperthyroidism with x-ray, one would expect this means to be more in use than is actually the case. Pfahler (72), who can be considered the pio-

neer in this field, and others (13, 73–76) have demonstrated clearly the real value of x-ray therapy. As early as 1917 Means and Aub (77) were able to report favorable results, and in 1924 Holmes, Means and associates obtained cures in one-third of their cases and improvement in another third. Despite this fact, x-ray has been used hardly at all at Means' clinic since.

Pfahler and Vastine (78) obtained 86% satisfactory results in their series, and in a statistical study of over 10,000 cases, Menville (79) reports 66% cure, 21% improvement and 13% failure. Harris and Rose have defined in a classical manner the place of x-ray therapy in the treatment of hyperthyroidism and claim as special advantages: *a*) negligible mortality, *b*) lack of major complications, *c*) low cost, *d*) absence of physical as well as psychic shock and *e*) maintenance of patient's earning capacity during treatment in most cases. They found satisfactory results in 67% of cases that followed the prescribed plan. European literature is almost entirely favorable and British, German and French authors report figures similar to the American studies. For example, Poulton and Watt (80) obtained marked improvement or cure in 55%, definite but incomplete improvement in 35%, whereas Hess (81) found 47% and 31% respectively in the same classification.

Herrnheiser and Redisch (82, 83) published a report of part of their material in 1927 and again in 1929, and in about 200 cases their percentage cure was the same as in Means' series. They were particularly interested in ascertaining whether or not x-ray gave the same favorable results after iodinization as before and found that previous iodine administration had apparently no influence on the results except in a small group of patients in which the effects were even better. They concluded therefore, that x-ray was indicated when insufficient improvement was obtained with iodine alone. Their technic of treatment was to employ a series of 8 to 10 consecutive weekly irradiations of 80 to 120 r, with rest periods of 1½ to 6 months depending on symptoms. Two or 3, even to 5 and 6 series, may have to be applied, and x-ray shock, which is essentially the same as a postoperative storm (84) is avoided in this way. Modern American technic is similar to the above. Adhesion of the capsule to the gland is no longer considered a result of irradiation (85–87).

Borak (88) employed x-ray to the temporal fields in addition to the thyroid with special improvement of the nervous symptoms. Whether this is due to action on the pituitary or on the vegetative centers of the diencephalon, we cannot say.

Radium has no advantage over x-ray (89) but may often be used more conveniently in emaciated patients confined to bed (90, 91).

The new method of administering radioactive iodine as developed in Means' clinic and presented by Hertz, might represent an effective and practical combination of the action of iodine and rays.

SUMMARY

Conservative therapy has its place in the treatment of hyperthyroidism because: *a*) of a certain number of surgical failures, *b*) certain cases are not amenable to surgery, *c*) mild cases generally do not need surgery at all.

The two conservative measures, generally complete in themselves, are: *a*) treatment with iodine, *b*) treatment with x-ray.

Administration of serum containing the hypothetic 'thyroid inhibitory' factor is discussed and regarded as a possibility for the future. Investigations in this direction are in progress.

Treatment with iodine alone is discussed with: *a*) cures in 10% of the favorable cases, *b*) no symptoms on iodine in 40%, *c*) incomplete but definite improvement in 50%.

The differentiation of cases on a constitutional basis is discussed. Iodine should never be given to patients with old nodular goiters become toxic.

Brief discussion of indication for surgery and of susceptibility to iodine poisoning in goiter prophylaxis is given. The technic of iodine administration is described.

X-ray treatment is described. Results in American literature surpass those in European reports. Previous iodinization is no contraindication to x-ray. Combination of both may even improve the results.

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A CASE OF ACROMEGALY STUDIED DURING THE COURSE OF THE DISEASE AND AT AUTOPSY

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SINCE THE monograph by Cushing and Davidoff (1) on acromegaly there have been only a few reports of this disease in which the clinical observations have been correlated with the autopsy findings (2, 3) as the patients are seldom seen terminally by those who have observed the case during life. The patient in this instance was seen 2 years after she had developed acromegaly and was followed in the hospital and clinic for the remaining 3 years of her life. During this time insulin sensitivity studies were made.

Acromegaly is one of the few well defined clinical conditions which can definitely be related to the hypersecretion of a hormone of the anterior pituitary. Furthermore, when injected into experimental animals, this hormone will produce the bony changes characteristic of the disease (4). Accompanying the bony changes there are other changes which may or may not occur in patients with acromegaly. Among these is diabetes mellitus, which is present in about 10% of the cases (5, 6) and was present in this particular case. The recent production of permanent diabetes in the dog by Young (7) by the injection of an extract of the anterior pituitary has added more evidence to the relationship of this gland to carbohydrate metabolism. The diabetes produced by Young in dogs differs in certain respects from the type of diabetes produced by extirpation of the pancreas, so that the possibility of pituitary as contrasted to pancreatic diabetes presents itself for consideration. In this patient the diabetes mellitus resembled in certain respects the type of diabetes produced by Young in dogs, the glycosuria was marked but acetone was never excreted in the urine and the glycosuria was not proportional to the amount of insulin injected.

Among the other symptoms that may occur in acromegaly are sexual impotence, irregularity or cessation of menses and disturbances of vision. All of these occurred in this patient.

CASE HISTORY

The patient, a 34-year-old white female, came to the Third Medical Division Diabetic Clinic of Bellevue Hospital on August 13, 1935. Her family history and past medical history were irrelevant. She had had one pregnancy, which was normal, at the age of 22. Her chief complaints at the time of admission were amenorrhea 'sugar in the urine' and enlargment of the face, hands and feet. The onset of these complaints was in 1933 when the patient noticed an increase in the size of her shoes from size 4½ to 6. In 1934 there was a loss of libido which had persisted. In February 1935 her menses ceased completely. In the 6 months prior to admission to the clinic the enlargement of the face, hands and feet had progressed and there also occurred a marked growth of hair on the extremities and face. Polyuria, polydipsia and polyphagia began early in 1935 and the presence of glycosuria was confirmed in another hospital at the time these symptoms occurred.

The patient entered the hospital for study and treatment on August 14, 1935. On physical examination she appeared chronically ill but in no acute discomfort. Her weight was 120 lb. She presented the classical acromegalic facies with marked enlargement of the nose and mandible and malocclusion of the teeth of the upper and lower jaws. There was a firm stubble of beard over the chin and upper lip. The pupils were slightly irregular, equal in size and reacted to light and accommodation. The fundi showed marked thinning of the arteries but there was no arteriovenous compression, hemorrhage or exudate. The teeth were carious and many were missing. The tongue was enlarged, thick and fissured in the midline. The pharynx was normal. The thyroid was not palpable. The lungs were clear. On examination of the heart the PMI was found in the 5th intercostal space at the midclavicular line. The sounds were of good quality. A_2 was greater than P_2. There were no thrills or murmurs. The rhythm was regular. The rate was 92 per minute. The blood pressure was 180/118. The abdomen was soft and flabby. The liver was palpable 1 finger below the costal margin. There were no other masses. There were no abnormal neurological findings. The hands and feet were very much enlarged, with thick stubby fingers and toes and moderate hypertrichosis. The pubic hair was normal in distribution and there was no abnormal growth of hair on the trunk. Gynecological examination showed hypertrophy of the labia minora and enlargment of the clitoris (¾ inch). X-ray examination showed an enlarged sella turcica. The clinoids were intact. Intravenous pyelograms were normal. X-ray of the heart showed slight enlargement. Examination of the visual fields showed uniform bilateral contraction. The basal metabolic rate was +19%. The first 24-hour urine specimen showed 4.5% sugar (51 gm.) but no acetone. The glycosuria was controlled after 7 days with a diet of carbohydrate 180 gm., protein 65 gm. and fat 65 gm., and 73 U of insulin daily in divided doses.

The diagnosis of an eosinophilic tumor of the pituitary with acromegaly and diabetes mellitus was made. Operative removal of the tumor was advised but refused by the patient. She was discharged to be followed in the clinic, on October 11, 1935.

In view of the fact that the patient refused operation, x-ray therapy to the region of the pituitary was begun at this time. From October to December 1935, the patient received a total of 1800 Roentgen units over each frontal region, the forehead and vertex.

In December 1935 the patient began to complain of severe headaches and failing vision. From this time on until her death in April 1938, her course was steadily downhill and was characterized by headaches of increasing severity, failing vision which progressed to almost complete blindness and an increase in the acromegalic features. During the period from August 1935 to April 1938 the changes that occurred have been summarized under the following headings: musculo-skeletal, eyes, cardiovascular and carbohydrate metabolism.

During the period observed the patient was admitted to the hospital 4 times and was seen in the clinic 76 times.

Musculo-skeletal system. Throughout the period of observation there was a slow progression of the skeletal changes. These were confined to the head, hands and feet. Osteoporosis did not occur. The sella turcica, which was enlarged on the first x-ray examination, did not increase in size. It measured 20 mm. in the anterio-posterior diameter. During the last year of the patient's life weakness and extreme fatigue were present. This intense fatigue is quite characteristic of the disease.

Eyes. The changes in the visual field, as already mentioned, were present in 1935, consisting then of bilateral contraction. In December, 1935, with the onset of failing vision the discs were found to be sharply outlined, the veins full and several flame shaped exudates and white patches were noted. The arteries were narrow and mod-

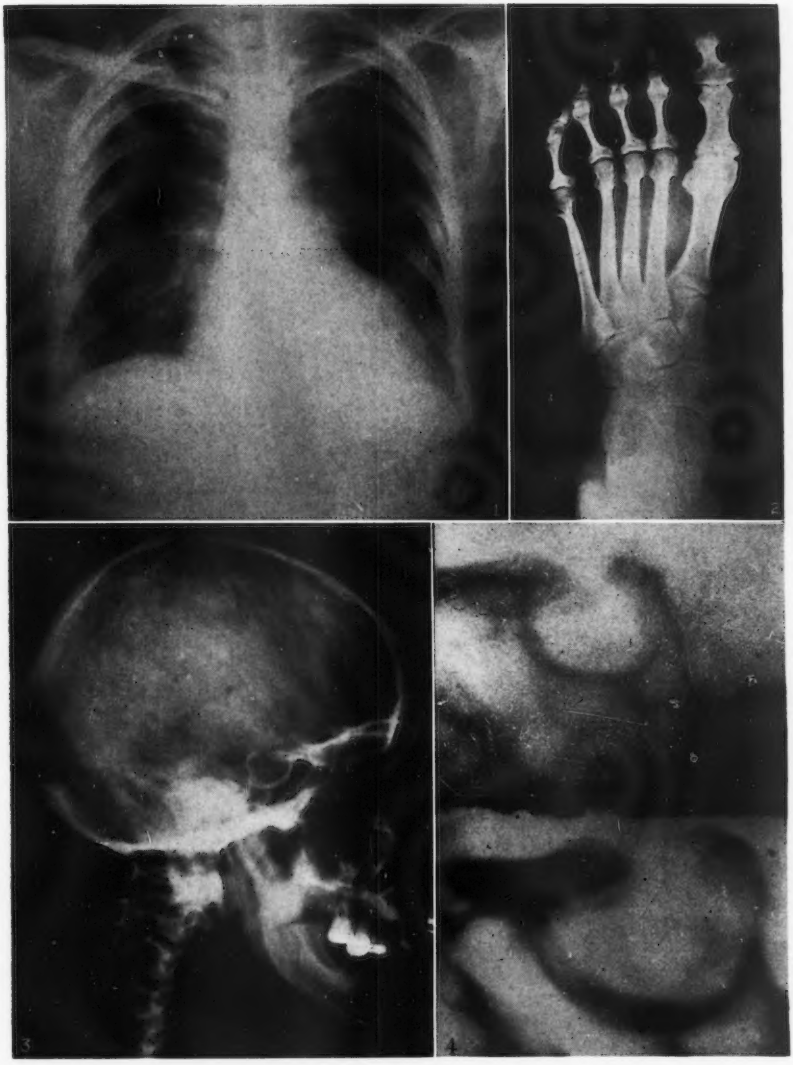

Fig. 1. TELEOROENTGENOGRAM SHOWING CARDIAC ENLARGEMENT, CHIEFLY LEFT VENTRICULAR. Fig. 2. X-RAYS OF FEET, SHOWING CLASSICAL TUFTING OF DISTAL PHALANGES. Fig. 3. LATERAL X-RAY OF SKULL SHOWING ENLARGED SELLA TURCICA. Fig. 4. SELLA TURCICA OF THIS PATIENT (lower), COMPARED WITH NORMAL SELLA (upper). SAME MAGNIFICATION.

erately tortuous. The failing vision progressed so that by April 1938 the patient could only distinguish light and dark. The last examination revealed bilateral lens changes with vitreous opacities, diffuse retinal hemorrhages and white exudates. There was detachment of the retina in the right eye.

Cardiovascular system. The blood pressure remained elevated throughout the course ranging from 180/110 to 235/170. The heart showed progressive enlargment

by x-ray. There were no signs of congestive heart failure nor were there any complaints due to diminished cardiac reserve, but her activity was, of course, limited because of her failing vision. Electrocardiograms showed a left DEA, slurring of the QRS, and inverted T wave in Lead I. Kidney function tests made one month prior to death showed a maximum specific gravity of 1.030 with a diluting power to 1.005. There was only a slight diminution of urea and phenolsulphophthalein excretion.

Carbohydrate metabolism. On her first admission to the hospital in August, 1935 the urinary glucose was 4.5% or 51 gm. in 24 hours but there was no acetone. The patient remained in the hospital on this visit for 29 days and received a diet of carbohydrate 180 gm., protein 65 gm., and fat 85 gm. Insulin was injected daily and when the dose was increased to 73 U daily the patient was kept practically sugar free. One week later when she reported to the clinic the urine again showed 4%

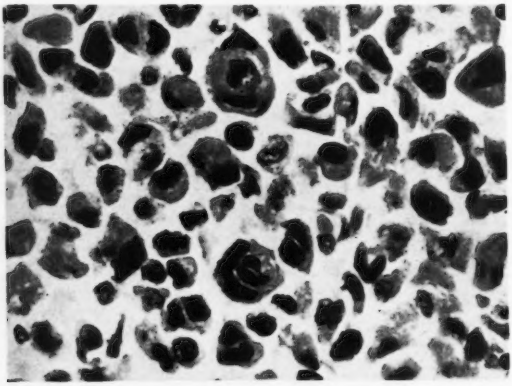

Fig. 5. PHOTOMICROGRAPH OF SECTION OF ADENOMA OF PITUITARY GLAND, SHOWING EOSINOPHILE OF VARYING SIZE AND SHAPE AND NUCLEAR CHARACTERISTICS.

glucose and no acetone. For the next 3 months, in which time there were 11 clinic visits, the urine continued to show from 2 to 6% sugar. During this time the daily insulin dosage was raised to 120 U, one dose being given at bed time. On the 11th visit the patient was sugar free, but returned a week later with glycosuria. The daily insulin was gradually increased to a total of 180 U but a glycosuria of about 2% persisted, although acetone was never found. This glycosuria was not accompanied by any loss of weight. The patient was not following the diet strictly but her inconsistencies in this respect were consistent. A venous glucose tolerance curve made in November 1935 showed a fasting blood sugar of 347 mg.% which rose ½ hour after the injection of 50 gm. of glucose to 500 mg.% and after 3 hours had only fallen to 333 mg.%.

In April 1936 the patient was again hospitalized. On admission this time the urinary sugar was 2%. The patient was placed on the same diet as before and 180 U of insulin in 4 doses. The glycosuria was controlled in 24 hours and the patient left the hospital after one week. After this she returned to the clinic and until August 1936 was relatively sugar free on this dose of insulin. The urine specimen of August 20th again showed sugar and the insulin was raised to 200 U daily. This did not control the glycosuria and the patient was hospitalized for the third time on October 15, 1936. On admission the urine contained 2% sugar and this was controlled on the same diet of carbohydrate 180 gm., protein 65 gm. and fat 85 gm., and 200 U of insulin.

The insulin was then gradually reduced and on November 11th was discontinued. For 22 days the urine remained sugar free and then began to show 1 to 2% sugar. No insulin was given however, for another 24 days but by then the glycosuria had reached 75 gm. daily and insulin was begun, starting with 45 U daily. This was raised daily and by the 7th day the patient was receiving 95 U and was again sugar free. She was discharged 10 days later on this dose. From this time on until her death the patient was never sugar free on her clinic visits and undoubtedly her inability to follow the diet influenced this to some extent, but obviously this was not the only factor. To check this she was hospitalized again in February 1938 and the insulin

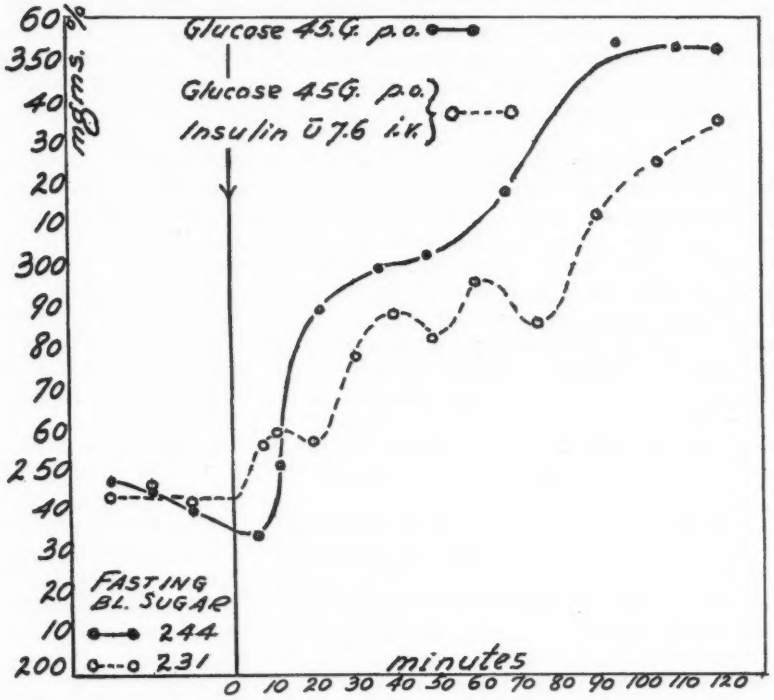

Fig. 6. CUTANEOUS BLOOD SUGAR CURVES. Solid line, curve following 45 gm. glucose by mouth; broken line, curve following 45 gm. glucose by mouth and 7.6 U insulin intravenously.

was decreased from 180 U to none in 6 days. The average daily urinary excretion for 6 days on no insulin was about 6 gm. From this time on in the clinic we kept her on 50 U daily. This was only for a period of 2 months, however, as she died in April, 1938. Apparently the glycosuria could be controlled by diet and insulin and when this had been done the patient could continue without insulin for varying periods of time. Regardless of the degree of glycosuria, acetonuria was not present, which is interesting in view of the very large amounts of sugar that the patient excreted for long periods of time.

During the third hospital period while the patient was sugar free and was receiving no insulin, detailed capillary blood sugar studies were made by the method previously described (8). The first curve (fig. 6) made after the ingestion of glucose alone showed a high fasting blood sugar and this continued to rise during the 2 hours

of the test. In the second blood sugar curve, made after the ingestion of glucose and the simultaneous intravenous injection of a standardized dose of insulin the blood sugar levels were as high as when insulin was not given. The insulin administered had apparently no effect in controlling the hyperglycemia. On the basis of these insulin sensitivity tests the diabetes would be classified as insulin-resistant or insulin-insensitive. This type of reaction is in striking contrast to that obtained in normal individuals or in the uncomplicated young severe diaebtic who responds readily to insulin.

Other laboratory data on the patient were not abnormal. On April 16, 1938, the patient developed tonic and clonic convulsions and became unconscious. On examination she was found to have active tendon reflexes and a negative Babinski. The blood pressure was 300/160. Lumbar puncture showed a crystal clear cerebrospinal fluid. The blood sugar was 420 mg.%; the blood non-protein nitrogen was 42 mg.%. There was 4+ sugar in the urine and no acetone. She died after being comatose for 36 hours.

On microscopic examination the pituitary gland consisted mainly of the anterior lobe. Hematoxylin and eosin stained sections showed that the cells were almost exclusively eosinophiles (alpha cells). There were a few chromophobic cells but these were very sparse. The eosinophiles themselves varied in size and contained a smooth hyaline cytoplasm in which no granules could be made out. There were also many cells with single nuclei which varied in size and shape from cell to cell. In addition many bizarre nuclear structures and multinucleated cells with 2 to 5 nuclei were encountered. These nuclei were vesicular or pyknotic, oval or spindle shaped. Some cells contained clear droplets in their cytoplasm and some showed marked vesicular degeneration of their nuclei. Basophilic cells were conspicuously absent. This was substantiated in sections stained with Mallory's Blue Orange G. Microscopic examination of the pars intermedia showed nothing remarkable. The diagnosis of an 'Eosinophilic adenoma with regressive nuclear changes' and 'hyaline degeneration' of the eosinophiles was made.

Sections of the ovary showed several primordial follicles, corpora albicantia and there was one partly maturated follicle showing cystic dilatation and scanty proliferation of the theca interna. The kidneys showed a moderate degree of arteriolar nephrosclerosis. The adrenals appeared fairly normal. The medulla was normal. There was slight lipoid storage.

DISCUSSION

This patient was not clinically an uncomplicated case of acromegaly. The most serious complicating factor was the hypertension. This was pronounced and persisted during the 3 years that she was under observation. It was the opinion of the ophthalmologists that the retinal changes and loss of vision were due to the hypertension. It seems probable, furthermore, that the hypertension was the precipitating factor in the patient's death. Courville and Mason (9) feel that when hypertension does occur in acromegaly, it should not be considered a part of the disease. Apparently the eosinophiles per se do not secrete a pressor substance as hypertension was not present in the majority of cases of acromegaly reported. Atkinson (10) in his monograph only lists this complication in a few cases. Courville and Mason (9) in 24 cases of acromegaly report 3 with hypertension. On the other hand in basophilic tumors of the anterior pituitary hypertension is the usual finding and is considered a part of the syndrome.

Diabetes mellitus, as already mentioned is not an uncommon finding in acromegalics. In this case the glycosuria was severe. It seems highly probable judging from the course of the diabetes e.g. the absence of ketonuria in spite of marked glycosuria,

the insensitivity to insulin as measured by insulin sensitivity tests and the fact that the glycosuria was not proportional to the amount of insulin injected daily even when the diet was controlled, that an extra pancreatic factor was responsible for the glycosuria. The findings at autopsy of an adenoma of the pituitary consisting solely of eosinophilic cells seems to provide such a factor. The similarity of the diabetes in this patient to the type of diabetes produced by Young in dogs furthers consideration of a pituitary type of diabetes as distinguished from the pancreatic type. The former is apparently not as severe in that it is not associated with the disturbance of fat metabolism that is such a frequent complication in pancreatic diabetes both clinically and experimentally in dogs.

In this patient pathological examination of the other endocrine glands, with the exception of the ovaries, did not show any significant changes. The ovaries were atrophic which undoubtedly accounted for the cessation of menses. This absence may have been related to the absence of the basophilic cells.

The presence of normal adrenal glands in this patient helps to limit the essential pathology to the hypophysis. This is of particular interest because of the interrelation of the pituitary and the adrenal cortex. Lukens and Dohan (11) found that adrenal cortical extract is capable of restoring the diabetes of the adrenelectomized, depancreatized animal. Therefore in this case one might suspect that the secretion of the eosinophilic cells was capable of producing diabetes because the adrenal cortex was not abnormal. In a recent case of acromegaly reported by Moehlig (3) diabetes was not present and at autopsy there was unilateral adrenal hyperplasia with hypertrophy of the cortex. The pituitary tumor in this case consisted of chromophobe cells which replaced the eosinophilic and basophilic tissue. In the experimental animal there are fewer complicating factors and the results are therefore more clear cut. Clinically one can still only speculate as to the course of events.

There was little clinical or pathological evidence in this patient that the course of the disease was influenced by x-ray therapy. The case reported by Moehlig was also treated with x-ray and with apparently no demonstrable effect on the course of the disease. In view of such findings in two cases both followed clinically and examined post-mortem, it would seem that surgical removal of the tumor when feasible was the better method of therapy.

Our findings in this case confirm those of other observers that acromegaly is a syndrome due to a disturbance predominantly of the eosinophilic cells of the anterior pituitary. The other features which may accompany the bony changes will depend on the other functions of the pituitary gland that are disturbed or on the involvement of other glands of the endocrine system. In addition patients may have some symptoms for which at the present there is no satisfactory explanation.

We are indebted to Dr. Irving Graef of the Department of Pathology for the descriptions of pathological specimens included in this report.

Due to necropsy restrictions the postmortem observations were limited to the findings recorded above.

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THE INHIBITION OF LACTATION POST-PARTUM WITH TESTOSTERONE PROPIONATE[1]

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INHIBITION OF LACTATION in the human being by means of testosterone propionate, was first reported by Kurzrok and O'Connell (1). 'Drying-up' of the secreting breast was effectively accomplished without special nursing care, and with little or no discomfort to the parturient. This report has led us to study the day post-partum and effective dosage which will produce optimal results.

The association of hypophysis with lactation, first noted by Evans and Long (2), and subsequently demonstrated in ablation experiments, by Selye et al. (3), Gomez and Turner (4), Hill (5) and the work of Riddle and coworkers lead to our present knowledge of anterior pituitary gland control of lactation and isolation of its fraction, generally identified as prolactin (6, 7, 9). The view, now generally held, is that preparatory changes in the hypophysis and in the breast tissue are initiated and maintained throughout pregnancy, and these are intimately associated with the hormone mechanism functioning after nidation has taken place (8). Secretion of breast milk appears subsequent to the alteration of the hormone balance following parturition. Inhibition of lactation occurs when lactogenic stimulus is inhibited or wanting (8).

Interruption of the hormone balance with the object of inhibiting lactation, post-partum, have been attempted repeatedly. The barriers are our incomplete knowledge and inability to produce a pure hormone which will effectively induce it. Synthetic estrogen and androgen compounds have more recently been tried, in animal research and clinically. The impression has been gained that effects due to inhibition of the hypophysis followed when testosterone propionate was administered to animals and to man.

Bates et al. (9) have observed pronounced antigonadal effects in birds treated with related sex hormone preparations. Thus, the effects of treatment with testosterone and related compounds were similar to those obtained by hypophysectomy. Rapid and extensive atrophy of the gonads and suppression of the follicle-stimulating hormone followed. Mazer and Mazer (10) noted temporary but marked ovarian atrophy and consequent utero-vaginal effects, suppression of estrus, decrease in size of adrenals in the mature rat, and decrease in size of the pituitary gland in the immature rat, following prolonged treatment with testosterone propionate. Restitution of structure and function returned after discontinuance. The ovarian and consequent effects were considered as secondary to pituitary inhibition. Zuckerman (11) produced similar effects in the monkey, Foss (12) and Loeser (13) in woman, by suppressing the menses; arrest of endometrial growth, and consequent vaginal effects. Numerous reports (12, 13, 14) have been published of suppression of menses following administration of male sex hormone in cases of menometrorrhagia and chronic cystic mastitis, and Papanicolaou et al. (14) have shown decided change in cytology, comparable with low estrin phase in vaginal smears.

[1] We are indebted to Dr. R. MacBrayer of the Ciba Pharmaceutical Company for their generous supply of perandren, testosterone propionate, used in this study.

TABLE 1. INDICATIONS FOR ADMINISTRATION OF TESTOSTERONE PROPIONATE

A. Maternal—82 cases	
Anatomical malformations or inflammation of breast or nipples	36
Economic and social reasons	6
Maternal cachexia, poor quality of milk	18
Excess milk	1
Toxemia	3
Morbidity, general	6
Miscarriage (6 months)	2
Cardiac disease	6
Colitis	1
Nervous disorder	1
Maternal allergy to milk	1
Contagion, pertussis	1
B. Fetal—25 cases	
Stillbirths	15
Neonatal deaths	5
Prematurity	3
Morbidity	2

Suppression of gonadotropic properties in the human being by synthetic androgens has been reported by Hamilton and Wolfe (15). The increase of gonadotropic capacity subsequent to castration and in the menopause is in a great measure offset by injections of testosterone propionate.

Histological changes in the hypophysis with altered function exhibiting suppressing or inhibiting effects when synthetic sterols and related compounds were administered to animals and in man, have been reported by Hamilton (15, 16). Relatively different structural changes follow treatment with estrogens and androgens. Injections of estrogenic substances cause degranulation of many or all of the basophils and to a lesser extent of eosinophils, with a concomitant increase of the chromophobes and a reduction in the percentage of the chromophils. Androgens produce only degranulation of the basophils.

Our report of the action of testosterone propionate upon lactating breasts, is based upon a study of 108 consecutive cases referred for 'drying-up' of the breasts, post-partum, from the Menorah Maternity, Beth-El Hospital. Results following administration of testosterone propionate were grouped as follows: (*a*) optimum result, when complete inhibition of lactation followed, (*b*) partial result, when milk secretion was not inhibited, but was appreciably diminished; Relief from engorgement or distension and pain or discomfort, in the lactating breasts, was the usual accompaniment with both of these results, (*c*) failure, when no appreciable influence upon the breasts or symptoms were noted.

TABLE 2. *Group I*, 3 INJECTIONS OF 25 MG., 12-HOUR INTERVALS

Began injection on day post-partum	No. Cases	Optimum	Partial	Failure	Not tabulated
1	2	2	0	0	0
2	6	5	0	1	0
3	21	15	6	0	0
4	11	4	6	0	1
5	10	8	2	0	0
6	3	3	0	0	0
8	8	5	2	0	1
9	1	0	1	0	0
10	2	1	1	0	0
14	1	0	1	0	0
Total	65	43	19	1	2
Per cent		66%	29%	1.5%	

TABLE 3. *Group II*, 4 INJECTIONS OF 25 MG.[1]

Began injection on day post-partum	No. Cases	Optimum	Partial	Failure	Not tabulated
1	7	2	5	0	0
2	3	2	1	0	0
3	16	8	7[2]	1[2]	0
4	5	1	4	0	0
5	1	1	0	0	0
6	1	0	1	0	0
7	3	0	2	0	1
Total	36	14	20	1	1
Per cent		39%	56%	3%	

[1] Thirty-four cases received 4 injections of 25 mg., the first 3 doses at 12-hour intervals, final dose, 6 hours later.

[2] One case in this group received 2 injections of 25 mg., and 2 injections of 10 mg.

Three cases in this group were not tabulated for lack of data.

Testosterone propionate was injected intramuscularly, and 3 injections of 25 mg. each, were administered, at 12-hour intervals. Review of the first group of treated cases suggested a change in dosage and variation in schedule of injections. The objective was to increase the number of optimal results from a large group exhibiting only partial effects. Subsequent cases were separated, having received additional amounts of testosterone with variation in the method of administration. All cases were classified into the 3 groups which follow.

DISCUSSION

Lactation, post-partum, has long been associated with hypophyseal control which follows parturition with expulsion of placenta and elimination of the chorionic source of inhibiting influence. This hormone mechanism has gained further substantiation in recent years, with increasing knowledge relating to the physiology of the reproductive system during pregnancy and lactation, and hormone excretion studies. The clinical results adduced with testosterone propionate seem to be related to inhibitory effects of anterior pituitary as a result of structural and functional alterations.

The therapeutic rationale of male sex hormone in 'drying-up' breasts, post-partum, may be inferred from the foregoing behavior, as noted in animal research and from clinical observation. It appears that it is effective by the changes it can and may

TABLE 4. *Group III*, VARIABLE DOSAGE AND SCHEDULE

Began injection on day post-partum	No. Cases	Optimum	Partial	Failure	Treatment	
					No. Inj.	Dosage
2	2	2	0	0	5 of	25 mg.
3	1	0	1	0	2 of	25 mg.
	1	1	0	0	1 of	25 mg.
					1 of	10 mg.
	1	1	0	0	1 of	10 mg.
4	1	0	1	0	1 of	10 mg.
10	1	0	1	0	1 of	10 mg.
Total	7	4	3	0	12	
Per cent		57%	43%			

induce in the pre-conditioned hypophysis, both structurally and functionally—an alteration which not alone inhibits, but also precludes the return of the lactogenic function until the end of another period of gestation.

One is impressed with equally effective results obtained in our study, before and after the third post-partum day, and later in the puerperium; variability in quantities

TABLE 5. A COMPOSITE TABLE OF 105 CASES TREATED (TABLE 2 TO 4)

Began inj. on day post-partum	No. Cases	Optimum	%	Partial	%	Failure	%	Treatment No. Inj.	Dosage
1	2	2		0		0		3	of 25 mg.
	7	2		5		0		4	of 25 mg.
			44%		56%				
2	6	5		0		1		3	of 25 mg.
	3	2		1		0		4	of 25 mg.
	2	2		0		0		5	of 25 mg.
			81%		9%				
3	21	15		6		0		3	of 25 mg.
	14	8		6		0		4	of 25 mg.
	1	0		1		0		2	of 25 mg.
	2	0		1		1		2	of 25 mg.
								2	of 10 mg.
	1	1		0		0		1	of 25 mg.
								1	of 10 mg.
	1	1	63%	0	35%	0		1	of 10 mg.
4	10	4		6		0		3	of 25 mg.
	5	1		4		0		4	of 25 mg.
	1	0		1		0		1	of 10 mg.
			31%		69%				
5	10	8		2		0		3	of 25 mg.
	1	1		0		0		4	of 25 mg.
			82%		18%				
6	3	3		0		0		3	of 25 mg.
	1	0		1		0		4	of 25 mg.
7	2	0		2		0		4	of 25 mg.
8	7	5		2		0		3	of 25 mg.
9	1	0		1		0		3	of 25 mg.
10	2	1		1		0		3	of 25 mg.
	1	0		1		0		1	of 10 mg.
14	1	0		1		0		3	of 25 mg.
Total	105	61		42		2			
Per cent		58%		40%		2%			

necessary to attain inhibition; and the irreversible status, once inhibition of lactation was induced with testosterone propionate.

No deleterious effects from the administration of testosterone propionate have been observed. Puerperal involution with subsequent return of the menses was unaffected. Obvious breast tissue changes of palpable nodules or cysts have not been seen.

Androgen excretion (17) in normal males shows that augmenting excretion values

are attained rapidly with increasing amounts of androgens administered. Relatively small quantities are apparently retained for utilization, and comparatively small quantities are required to be effective in the body. Our studies[2] of androgen excretion of two parturients, before and after administration of androgens, indicate similar results. Effectual dosage depends upon the level of excretion of the individual rather than quantities administered. A high androgen titre is necessary to produce the structural and functional alterations which will effect inhibition of lactation.

A large number of the group studied yielded only partial inhibition, and varying the dosage and the schedule of injections did not increase the number of optimal results. The number of cases completely inhibited was not apparently related to the post-partum date of beginning of treatment. Thus, 81% of cases in which treatment was begun on the 2nd or 5th day post-partum were completely inhibited, 63% of those begun on the 3rd day, and 32% of those on the 4th day. Effectual dosage seems to depend on rapidly attaining a high titre of androgens.

SUMMARY

A study group of 108 cases of the inhibition of lactation, post-partum, by means of testosterone propionate is presented. The effectiveness of the method is tabulated and summarized in a composite table of all cases with dosage and schedule of injections. Complete inhibition occurred in 58%, incomplete results in 40%. All these obtained relief from engorgement or distension and pain or discomfort in the breasts. Failure occurred in 2%.

The therapeutic rationale of testosterone propionate in inhibition of lactation post-partum is discussed.

A brief review of the physiology of the reproductive system and the hormone mechanism during pregnancy and lactation is presented.

We are deeply indebted to Dr. Raphael Kurzrok of the Presbyterian Medical Center, New York City, for his advice and assistance and to Dr. Mendel Jacobi, director of laboratories, for his helpful suggestions. The authors wish to express their appreciation to Dr. A. Koplowitz and members of the Obstetrical Division for their permission to carry out this study; to Edith Miller, R.N., supervisor, and nursing staff at the Menorah Maternity for their painstaking observations and cooperation in recording the data.

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[2] These will be reported in a separate communication.

MUSCLE STRENGTH IN MYOTONIA ATROPHICA (DYSTROPHIA MYOTONICA) IMPROVED BY TESTOSTERONE PROPIONATE

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An association between endocrine activity and the normal development and function of skeletal muscle has long been suggested. Chemical interrelationships of adrenal cortical substance with androgenic hormone and their rôle in electrolyte-fluid balance and creatine-creatinine metabolism have been studied (1, 2, 3). Some observers (4) have noted greater working efficiency in normal people given adrenal cortical extract, and the improved muscle function in similarly treated cases of Addison's disease is generally accepted. Others (5, 6) administered cortin in certain progressive myopathies with varying benefit.

Recently, androgenic hormone has been shown to cause dimensional and dynamometric increases in the general skeletal musculature of male castrates (7, 8) as well as a response in the diaphragmatic and bladder musculature (9). Definite muscular and psychomotor improvement has been recorded (10) in senile males similarly treated and excellent results obtained in the alleviation of asthenia in women (11). Experimentally, other observers (12) produced muscle hypertrophy in female and castrated male guinea pigs with androgenic hormone and suggested an applicability to conditions associated with muscular deficiencies. Myotonia atrophica is a myopathy associated with gonadal atrophy as well as degenerative changes in the adrenal cortex; hence an additional rationale for employing androgenic therapy exists in this disease.

Two male patients with myotonia atrophica and testicular atrophy were given 25 mg. of testosterone propionate (perandren)[1] intramuscularly every other day for over two months. These individuals had been studied (13) previously and were still taking quinine daily. The dynamometric response of the finger flexors was recorded graphically to note strength (single maximal contraction), total work, and maximal flexion rate before and during treatment. Total work was measured by performing maximal contraction every 2 seconds for 2 minutes, the initial and final values affording satisfactory comparative estimation with the intervening strokes creating a fatigue curve. Other subjective and objective changes accompanying treatment were carefully observed.

Results of treatment were evident within the week of the first injection. Thereafter the grip became stronger rapidly, reached a peak within the next 2 weeks, and leveled subsequently at the higher baseline. Temporary withdrawal of the drug in

[1] We wish to thank the Ciba Pharmaceutical Products Co. for their kindness in supplying the perandren used in these studies.

one case was accompanied by decreased dynamometric values 4 days after the last injection. Actual figures rose from 6 to 16 kg. strength in one case and from 8 to 16 kg. in the other. This marked a gain to more than twice the original level, although the new status was still approximately 25% of that for normal males. Total work was increased by more than half the original amount. There were no significant changes in contraction rate.

Both patients were gratified with the outcome of treatment, especially by the sense of greatly increased strength and the general constitutional improvement. Buoyancy of spirit, feeling of well-being and lessened fatigability were noteworthy. Posture and gait improved noticeably. A weight gain of some 15 lb. occurred; this might have been due to hydration [retention of electrolytes, nitrogen and water (3)], fat deposition (increased appetite), or actual muscle dimension changes (hypertrophy). Frequency of micturition correlated with previous observations on bladder musculature (9). Recurrent priapism with increased sex urge, as usual became evident.

Skill in the use of the dynamometer affected the values little if any. Dynamometric readings were made daily for 2 months by 6 normal controls without showing appreciable improvement in strength. Mental attitude and cooperation, grip position and time of day seemed to make for minor discrepancies, but average figures were constant. There was little variation in the consecutive values attained by the patients.

A third male patient given androgenic hormone without quinine showed no change in myotonia. Maximal contraction in this instance required a suitable warm-up period, and some improvement was manifest although the duration of treatment was short. Quinine alone has been shown to relieve myotonia without improving strength (13).

Another male patient with moderately advanced progressive muscular dystrophy but no gonadal changes was treated with testosterone propionate without demonstrable dynamometric response.

How androgenic hormone influences muscle activity is still unexplained. It is responsible for the greater muscular development and capacity of the adult male and its inadequacy certainly accounts for the well-known muscle alterations in male castrates. Credit must be given to the general muscular as well as tonic and psychomotor stimulation androgenic therapy produces in these cases. Here, in view of actual structural changes induced in the striated musculature by the hormone, the effect seems a predominantly mechanical one. However, considering its ability to cause electrolyte, nitrogen and water retention in these individuals, testosterone undoubtedly acts as a metabolic synergist in much the same manner as adrenal cortical substance.

Whether the dynamometric changes following androgenic therapy in myotonic dystrophy reflect improved function in the affected muscle fibers, a response in the remaining more normal musculature, or alteration in both cannot be ascertained from the above results. Since benefit seems to obtain only in myotonic dystrophy associated with gonadal atrophy and since no significant dynamometric changes occur in progressive muscular dystrophy showing no gonadal alterations, we feel the effect is probably a non-specific one that would occur in any castrate. However, this added improvement in strength over the initial marked weakness in these cases of myotonic dystrophy is obviously a proportionately greater benefit than would obtain in an ordinary castrate and therefore seems well worth producing.

In order to be more certain of the specificity and mode of action of testosterone propionate in myotonia atrophica, we are continuing treatment in these and other

cases, and further studies regarding changes in bodily structure, electrolyte and creatine-creatinine metabolism, and muscle histology are in progress. Its applicability in the treatment of women with myotonia atrophica is undergoing consideration. The rôle of the adrenal cortex is being investigated.

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Addendum. Since this communication was written the 2 cases described above were deprived of treatment and within the following month had reverted to their original dynamometric status.

FURTHER STUDIES ON THE PURIFICATION OF MARE GONADOTROPIC HORMONE

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AMONG THE METHODS used for separating the gonadotropic hormone from pregnant mare serum, the fractional precipitation method of Cartland and Nelson (1) has proved most suitable, in our hands, for consistently yielding fractions of high potency with low nitrogen and solids content. We are now reporting some experiments upon the purification of the hormone obtained from very potent sera from pregnant ponies, testing 100 to 400 rat units[1] per cc., together with some studies in which the starting material was obtained from less potent mare sera.

EXPERIMENTAL METHODS

The blood was collected from mares pregnant 65 to 75 days. The freshly drawn blood was defibrinated by whipping, and the serum obtained by centrifuging out the cellular constituents. Each batch of serum was worked up immediately; that is, treated according to the Cartland-Nelson process through the first precipitation of hormone by 70% acetone with the exception of two modifications. We first brought the original serum to PH 9 instead of PH 7.4[2] as suggested by Cartland and Nelson. The second modification was to add a volume of acetone equal to 95% of the volume of the serum instead of an equal volume. Both these modifications were employed in order to reduce the amount of hormone lost in the first precipitate.[3] After this first precipitate was taken off, the filtrate was brought carefully to 50% acetone by volume (sp. gr. 0.937 at 20° C.). It was then brought to PH 6 and treated as suggested by Cartland and Nelson. The 70% acetone precipitate was the starting point for the procedures to be described.

Effect of Varying the Concentration of Acetone Solution Used in Redissolving the Hormone Fraction Precipitated from 70% Acetone Solution

Cartland and Nelson recommended taking up the hormone in 10% acetone solution. We have found that by using higher concentrations, that is, up to 50%, we could recover most of the active material and leave behind some inactive material. The following experiments were carried out to determine what purification could be obtained by this simple procedure. Quantities of pregnant pony sera varying from

[1] The assay method used in this work is essentially that of Cole, Guilbert and Goss, (2): the dose was halved until the smallest dose was found which would produce an average of at least 3 corpora lutea or ripe follicles per rat. This dose was considered to be a rat unit. The ovarian weight method of Cole and Saunders (3) was helpful in finding this range.

[2] All PH determinations in both water and acetone solutions were made at 25° C. with a Coleman electrometer. No corrections were made for the presence of acetone. The instrument was standardized with a carefully prepared phosphate buffer in water solution.

[3] In 9 preparations of pony sera the recovery of hormone in the acetone filtrate at this point averaged 60%.

1,500 to 2,000 cc. were treated according to our procedure described above. The material finally separating from 70% acetone solution was taken up in an amount of acetone solution approximately equal to one-fifteenth the original volume of serum. The concentration of the acetone used for resolution varied from 20 to 50% (table 1). The pH varied from 6.5 to 7.3 in these unbuffered solutions. The insoluble residue, having been removed by centrifuging, was taken up with 100 cc. of saline solution for bioassay and for nitrogen determination.

Wishing to carry out further fractionation of the material in the acetone solutions, we took out none for assay at this point. Each subsequent fraction, however, was assayed for total R.U. and nitrogen was determined by the micro Kjeldahl method of Kirk (4), on all fractions, (excepting some of the most potent preparations).[4] We did not wish to sacrifice at this time so much of our purified preparations as would be needed for nitrogen determinations. In these cases, therefore, we estimated the total

TABLE 1. EFFECT OF REDISSOLVING HORMONE PRECIPITATES IN VARYING CONCENTRATIONS OF ACETONE

Preparation No.	Potency estimated[1] R.U./mg. N	Acetone concentration % by volume	Insoluble residue		Acetone solution		
			Total N mg.	Total R.U.	Total N estimated[2] mg.	Total R.U.[3]	Potency estimated R.U./mg. N
111	3,200	20	1.4	100	25.6	87,000	3,400
115	13,800	30	4.5	1,000	11.3	275,000	24,300
116	16,000	30	4.9	1,000	10.8	250,000	23,000
117	10,700	30	8.6	100	10.0	200,000	20,000
118	7,500	30	4.1	100	22.6	200,000	8,800
119	9,500	30	11.5	1,000	33.3	425,000	12,800
120	4,500	30	5.1	500	24.4	131,000	5,400
121	11,300	50	5.5	1,600	6.5	135,000	21,000
122	1,380	50	11.3	1,000	8.2	27,000	3,300

[1] This figure was computed from data in columns 4 and 5 and from the nitrogen and hormone content of all the fractions of the acetone solution.

[2] This figure represents the sum of the nitrogen appearing in all the fractions of the acetone solution. In some of the most potent fractions nitrogen was estimated from the total solids on the basis of the composition of similar fractions.

[3] This represents the sum of all hormone units appearing in the fractions of the acetone solution.

nitrogen from the total solids, using the factor 10% (see page 5). The sum of the total R.U. of all fractions, of the acetone solution, constitutes the figure for total R.U. in table 1, column 7. The sum of the total nitrogen of all the extracts and the residual insoluble nitrogen was used to calculate the potency of the original material in R.U. per mg. of nitrogen given in table 1, column 2. The potency of the extracted material was calculated similarly.

As table 1 shows, some purification has been obtained by redissolving the precipitated hormone in 30 to 50% acetone solution. The best purification resulted from using 50% acetone. Further experiments are needed, however, to show whether or not the inactive material left behind here would not be removed just as effectively in the iso-electric precipitation to be described next.

Effect of Precipitation in 50% Acetone Solution at Different Hydrogen Ion Concentrations (Iso-electric Precipitation)

The active solutions remaining from the experiment described above, having a volume of approximately 100 to 150 cc., were brought to 50% acetone concentration and then adjusted to the pH shown in table 2, using dilute hydrochloric acid. Pre-

[4] See table 3 for an example of results on fractions from one preparation.

cipitates forming were allowed to collect and settle overnight at 2° C. They were then removed by centrifuging and taken up in 100 cc. of distilled water for assay. The supernatant liquid in each case was then adjusted to the next pH shown in the table, and this product removed for testing. Finally, the remaining filtrate was assayed for its hormone content. In table 2 the percentage of total hormone removed at these various points is shown and the potency in R.U. per mg. (in those preparations where solids were determined) is shown immediately below this figure by figures in parentheses.

TABLE 2. PERCENTAGE OF TOTAL HORMONE PRECIPITATED FROM 50% ACETONE SOLUTION AT VARYING PH, AND THE CORRESPONDING POTENCY[1] IN R.U. PER MG. OF SOLIDS

70% precipitate used, preparation No.	Total number R.U.[2]	pH					Filtrate
		6.0	5.5	5.0	4.5	4.0	
112	175,000	0		71 (900)		29 (300)	0
113	350,000	0		40 (1660)		60 (2000)	0
111	87,000	0	15 (500)		55 (1400)		30 (600)
115	275,000	0	0		91 (7000)		9
116	250,000	0	10 (2300)		80 (4000)		10
117	200,000	0	0		100 (5000)		0
118	200,000	0		50 (2000)	50 (3000)		0
119	425,000	0	3	24 (1590)	70 (4200)		3
120	131,000	0		19 (600)	77 (1400)		4
121	135,000	0			63 (4200)	37 (1800)	0

[1] The potency (figures in parentheses) express the number of R.U. per mg. of solids in the various fractions separating.

[2] This figure represents the sum of all the R.U. appearing in precipitates and final filtrate.

Preparations 112 and 113 were preliminary trials used in determining the best points for precipitation. Table 2 may best be explained by describing in detail the procedure for one preparation, such as number 115. The 30% acetone solution of the hormone, containing a total of approximately 275,000 R.U. in 150 cc., was brought up to 50% concentration with acetone; and the pH was adjusted to 6.0. A small amount of precipitate forming here upon standing in the cold room was removed, assayed, and found to be inactive. The filtrate was next adjusted to pH 5.5, whereupon a second, inactive precipitate formed and was removed. The pH was next taken to 4.5. The precipitate forming here was removed by centrifuging and was taken up in 100 cc. of distilled water. Upon assay we found that 91% of the original hormone had precipitated between pH 5.5 and pH 4.5, with a potency of 7,000 R.U. per mg. of solids. The remainder of the activity was accounted for in the filtrate when acetone was added to a concentration of 70% and the small amount of precipitate tested. The nitrogen partition for these fractions is shown in table 3.

Total solids were determined in all the active solutions by evaporating 5 cc. samples in a vacuum desiccator at room temperature. By this procedure we found that none of the hormone activity was lost. The residue, therefore, was redissolved in 5 cc. of water and returned to the stock solution. Ultra-micro nitrogen determina-

tions were carried out on 3 of the most potent preparations, using the method of Bently and Kirk (5). The results showed 11.3, 9.6, and 10.0% nitrogen, respectively, in the dry material. All other micro-nitrogen determinations were carried out with the apparatus described by Kirk (4).

Table 2 shows that in 50% acetone solution, precipitation of the hormone begins at pH 5.5 and is practically complete at pH 4.5. If the acidity is carried beyond pH 4.0, we have observed the solution to become clear with the disappearance of the precipitate. The fraction precipitating between pH 5.5 and 4.5 had the greatest purity. To us this evidence indicates that the isoelectric point for mare gonadotropic hormone in 50% acetone lies between pH 5.5 and pH 4.5, and probably closer to pH 4.5. The potency of our best preparation (see No. 115, table 2) is approximately 7,000 R.U. per mg. of solids. Six of the best preparations gave potencies of 4,000 to 7,000 U per mg. solids—higher than any potency we have found reported in the literature. Assuming a nitrogen content of 10% for the purest preparations, based upon the 3 analyses mentioned above and upon Cartland and Nelson's report, we should then

TABLE 3. TOTAL NITROGEN IN FRACTIONS SEPARATED AT VARYING PH IN 50 % ACETONE (PREPARATION NO. 115)

Fraction	Nitrogen, mg.	% of total N
Residue, insoluble in 50% acetone	4.5	27
pH 6.0 precipitate (inactive)	1.8	11
pH 5.5 precipitate (inactive)	0.9	5
pH 4.5 precipitate (active)	3.6*	21
pH 4.5 ppt. filtrate (slightly active)	6.0	36
Total	16.8	100

* Estimated from total solids, using factor 10 per cent.

expect from 40,000 to 70,000 R.U. per mg. of nitrogen for our most potent preparations.

The biological properties of the purified preparations will be considered in another paper. The response obtained with these extracts when injected into the intact immature female rat cannot be distinguished from that obtained with untreated serum.

Gurin, Bachman and Wilson (6) have recently reported a purification of gonadotropic hormone of pregnancy urine of approximately 4,000 of their units per mg. solids. This degree of purity may be as great as that which we have obtained with mare serum hormone. Cartland and Nelson (1) worked with a mare plasma containing 5 to 7 of their R.U. per cc. Since their unit is stated to be approximately 10 times that employed by us, their starting material would contain 50 to 70 of our units per cc. as compared with the sera which we employed containing 100 to 400 U. per cc. Multiplying their figures by 10 we shall see that they obtained products from the first precipitation with 70% acetone assaying 2,000 R.U./mg. N. By reprecipitating, using 65% acetone pH 6, they increased the purity to 4,000 R.U./mg. N, about one-tenth the purity of our best preparations from pony sera. Their best preparation recorded contained 14,000 R.U./mg. N and was reported to contain 10% nitrogen. However, this single preparation was apparently not obtained by their standard procedure. Assuming, again, that our potent fractions contain approximately 10% nitrogen, based upon the 3 determinations previously stated, and using the potencies of the various starting solutions listed in table 1, the purification obtained by this precipitation in 50% acetone is 1.7 to 4.1-fold (table 4).

TABLE 4. PURIFICATION OBTAINED BY ISO-ELECTRIC PRECIPITATION OF 70% PRECIPITATE TAKEN UP IN 50% ACETONE SOLUTION

Preparation No.	Potency, R.U./mg. N		Purification
	Starting solution	pH 4.5 precipitate	
111	3,400	14,000	4.1
115	24,300	70,000	2.9
116	23,000	40,000	1.7
117	20,000	50,000	2.5
118	8,800	30,000	3.4
119	12,800	42,000	3.3
120	5,400	14,000	2.6
121	21,000	42,000	2.0

Effect of Precipitation at pH 6 in Different Concentrations of Acetone Solution

These experiments were carried out on a preparation, No. A46C, made from horse serum before the pony sera were available. This preparation consisted of a saline solution of a 70% acetone precipitate, prepared by the Cartland-Nelson method. It assayed 200 R.U. per cc. and contained 5 mg. of organic solids and 0.3 mg. nitrogen per cc. This potency of 660 R.U. per mg. N is quite inferior to the potency of preparations made from pony sera, where values ranging from 1,380 to 16,000 R.U. per mg. N were found in the first precipitation of active material.

Two hundred cc. portions of the hormone solution A46C were treated with acetone to bring the concentration up to the values shown in table 5. In each case the pH was adjusted to 6.0. The precipitate formed was allowed to collect and stand for 50 to 70 hours at 2° C. It was then removed by centrifuging for assay. Next, the supernatant liquid was brought up to 65% acetone, whereupon a second precipitate formed and was removed for testing. All the original hormone activity was accounted for in these two precipitates.

Table 5 gives the results of 10 such experiments in which the acetone was increased between 50 and 60%.

As this table shows, the hormone begins to precipitate at 52.5% and is completely removed at 59.0%. Portions, therefore, of the original solution A46C were brought to 52.5% acetone, pH 6; and the precipitate forming here was removed and discarded. Upon increasing the concentration to 59%, a fraction was obtained that assayed 2,000 R.U./mg. N. This procedure represents a 3 to 4-fold purification, starting with a horse serum preparation of rather low potency.

TABLE 5. RELATIVE AMOUNT OF HORMONE PRECIPITATED AT PH 6 FROM ACETONE OF VARYING CONCENTRATION. STARTING MATERIAL: 200 CC. OF PREPARATION A46C ASSAYING 200 R.U./CC., 660 R.U./MG. N

Test No.	Acetone solution, percentage by volume	Percentage total hormone precipitated
1	52.0	1
2	52.5	4
3	52.5	8
4	52.5	8
5	55.0	10
6	55.0	4
7	55.0	50
8	59.0	100
9	59.5	100
10	60.0	100

Proposed Method of Concentrating Mare Gonadotropic Hormone

In the light of these experiments, we wish to propose the following method for concentrating mare gonadotropic hormone, based partly upon the procedure of Cartland and Nelson.[5]

The serum is first adjusted to pH 9, and a volume of acetone equal to 95% of the original volume of serum is slowly added with vigorous stirring. The precipitated proteins are allowed to stand 12 to 18 hours at room temperature and then separated out by means of flannel filter bags in a press. The pressed precipitate is washed once with a volume of 50% acetone (sp. gr. .937 at 20° C.) equal to the original serum, again pressed, and discarded. The combined filtrates are carefully adjusted to 50% acetone concentration; dilute hydrochloric acid is added to bring the pH to 6.0; and the solution is then allowed to stand at 2° C. over night. The inactive precipitate forming here is best removed with a super-centrifuge. The filtrate is brought to 70% acetone concentration and allowed to settle for 2 to 4 days at 2° C. The 70% acetone precipitate is now removed by centrifuging and is taken up in a volume of 40% neutral acetone equal in volume to one-fifteenth of the original serum. The insoluble material left behind may carry down some active material (table 1), which may, if desired, be worked up with other less potent preparations. It is separated by centrifuging, and the clear filtrate carefully adjusted to 50% acetone concentration and pH 5.5. The precipitate forming here is removed and discarded. The pH is now adjusted to 4.5, whereupon the active material separates as a slight precipitate and, after standing in the cold, is removed by centrifuging. It may be taken up in distilled water and kept frozen, or it may be preserved as a powder.

SUMMARY

Some results of experiments are given on the purification of the gonadotropic hormone of mare serum, starting with very potent sera of pregnant ponies and also with less potent horse sera. If the first 70% acetone precipitate obtained by the Cartland-Nelson procedure is redissolved in 50% acetone, pH 6–7, a 2-fold purification may be obtained. When this 50% acetone solution of the hormone was further fractionated by increasing the acidity in steps, a very potent fraction was removed between pH 5.5 and 4.5, which contained 4,000 to 7,000 R.U./mg. solids. At pH 6.0, the hormone begins to precipitate at 52.5% acetone concentration, by volume, and is completely removed at 60% acetone concentration.

The authors wish to express their sincere appreciation of the assistance of Mr. A. N. Booth who carried out much of the chemical work reported here and who performed many of the biological assays.

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[5] Our method involves the use of blood serum rather than plasma as the starting point. Sera of any potency may be treated by this method, although our best preparations were obtained from sera testing from 100 to 400 R.U. per cc.

THE SOJOURN OF INSULIN IN THE BLOOD OF RABBITS AFTER THE ADMINISTRATION OF MASSIVE DOSES OF INSULIN[1]

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THE INJECTION of insulin in a fasting animal causes a maximum decrease of the blood sugar within 2 hours, but the depression of the blood sugar is manifested for a longer period of time. This prolonged effect may be associated with the continued presence of insulin in the blood stream or to its persistent action in other tissues after it has disappeared from the circulation. Horsters (1) subscribes to the latter view. An answer to this problem may be obtained if the presence of insulin in the blood stream can be determined; obviously, when sufficiently large doses are injected intravenously into comparatively small laboratory animals, it may be shown that extracts made from the blood of such animals contain insulin.[3]

MATERIAL AND METHODS

Each of 10 full-grown, unanesthetized rabbits received approximately 1000 U of insulin intravenously (50 mg. of insulin powder assaying about 20 U per mg.[4] dissolved in 5 cc. of distilled water). In 4 rabbits, the blood sugar was determined on blood obtained from the marginal ear vein, method of Folin and Wu (2), before and at frequent intervals after the injection of insulin. In 6 rabbits, a needle was inserted in the heart and 6 cc. of blood removed before and at intervals of 2, 6, 15, 30, 60, 90, 120 and 180 minutes after the injection of insulin, when this was possible. One cc. of blood was used for the determination of the blood sugar. The remaining 5 cc. was introduced into approximately 50 cc. of 70% ethyl alcohol and allowed to stand at least overnight. The precipitate was removed by filtration and washed with small quantities of 70% alcohol. The filtrate was evaporated to dryness at room temperature and the residue taken up in 5 cc. of distilled water. Seventeen such extracts were prepared from the heart bloods of 3 rabbits; these were injected subcutaneously into fasted, unanesthetized rabbits and the blood sugar determined on bloods obtained from the marginal ear vein before and at frequent intervals for 2 hours after the injection of the extract.

Two of the 6 rabbits subjected to heart puncture and insulin lived only 15 and 50 minutes, respectively, after the injection of insulin. At autopsy, these showed ex-

[1] Aided by a grant from the Helen R. Goldman Fund.
[2] Now residing in Evanston, Illinois.
[3] A rabbit weighing 3 kg. has approximately 300 cc. of blood (10% of the body weight). After the injection of 1000 U of insulin intravenously, assuming no insulin leaves the blood stream, 5 cc. of the blood should contain approximately 17 U of insulin.
[4] We are indebted to the Eli Lilly Company for a generous supply of powdered insulin. This material contains about 20 to 21 U per mg. which approximates very closely the international crystalline standard of 22 U per mg.

tensive pericardial hemorrhages. Two lived 135 and 190 minutes, respectively. One animal was found dead in its cage approximately 17 hours after the injection of insulin; one rabbit survived after being fed. Of the 4 rabbits receiving the same dose of insulin but without withdrawal of blood from the heart, 1 survived following the administration of large amounts of dextrose by stomach tube; the remaining 3 died.

Eight of the 10 rabbits were fasted for 20 to 24 hours before the injection of insulin. One rabbit was starved for 60 hours, while another was given food until the time of the experiment. When fed, the rabbits received Rabbit Chow[5] once daily; water was allowed in the cages at all times.

The validity of extracting insulin from the blood, according to the procedure described above, was determined by adding insulin to blood in vitro; extracts prepared from such bloods exerted definite hypoglycemic effects when injected into fasting rabbits. Extracts made from blood to which no insulin was added showed no demonstrable influence on the blood sugar (as shown by the control blood extracts from 3 rabbits before the injection of insulin).

RESULTS

Figure 1 is a composite chart showing the rate of disappearance of insulin from the blood as gauged by the hypoglycemic effect of 17 extracts made from the blood of

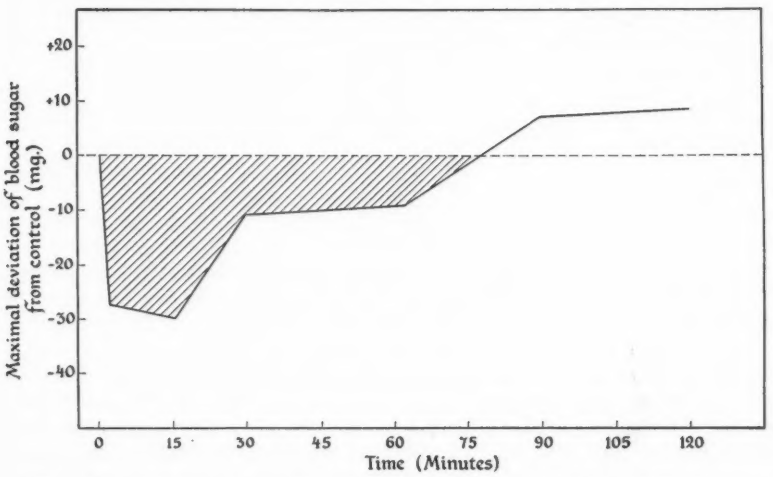

Fig. 1. Composite curve showing sojourn of insulin in the blood as gauged by the hypoglycemic effect of blood extracts prepared from the blood of rabbits which had received 1000 U of insulin intravenously.

3 rabbits, each of which had received 1000 U of insulin intravenously. The extracts made from blood of the rabbits prior to the injection of insulin failed to influence the blood sugar. The experimental extracts frequently caused a depression of 30 to 50 mg. in the blood sugar and at times hypoglycemic shock was produced in these test animals; this response was noted especially with the 2 and 15-minute extracts. The insulin effect was present to a lesser degree in the 30-minute extracts, although in one

[5] Prepared by Ralston Purina Co., St. Louis, Mo., and containing a mixture of grains and alfalfa hay supplemented by vitamins and minerals.

case a slight effect was seen in 90 minutes; the average duration, as judged from the curve, was about 75 minutes. If insulin did persist in the blood beyond 75 minutes, it had decreased in amount to the point that it was not detectable in extracts made from 5 cc. samples of blood. It is interesting to note that any change which occurred with the blood extracts taken after 75 minutes following the injection of insulin was in the direction of an elevation of the blood sugar above the control level.

Figure 2 shows the blood sugar curves of each of 9 rabbits injected with 1000 U of insulin intravenously and of 1 rabbit injected with 500 U. Strikingly enough, persistent and marked hyperglycemia occurred in 4 of the 9 rabbits injected with the

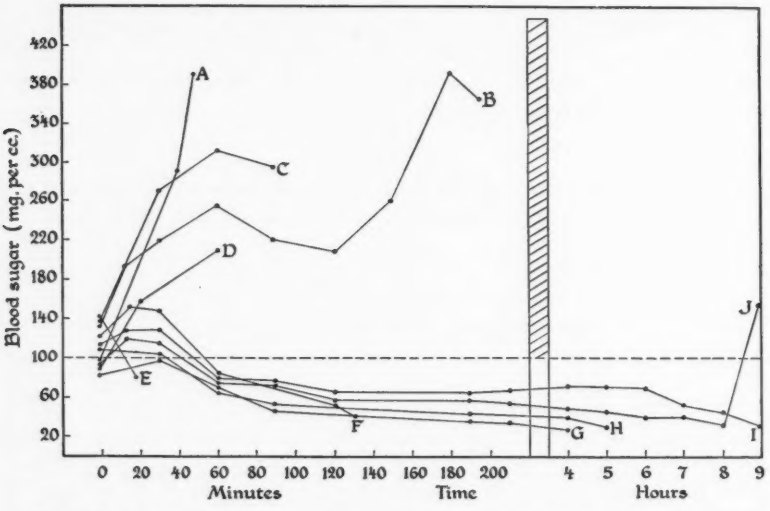

Fig. 2. BLOOD SUGAR CURVES AFTER THE INTRAVENOUS INJECTION OF 1000 U OF INSULIN IN RABBITS. *Rabbits A, B, D, E, F, G, H,* AND *J* DIED; *C* RECOVERED AFTER FEEDING; *rabbit I* RECOVERED AFTER DEXTROSE ADMINISTRATION BY STOMACH TUBE. (Blood sugar is expressed in mg. per 100 cc.)

larger dose of insulin. As mentioned above, one of these rabbits survived after being fed; dextrose was not administered. This animal manifested acetone in the expired air the following day (positive Scott-Wilson reaction); the urine was sugar-free. Four of the remaining rabbits showed a variable and transient hyperglycemia followed by hypoglycemia. One of this latter group recovered after dextrose was given by stomach tube. No hyperglycemia was observed in the rabbit receiving 500 U of insulin intravenously.

The hyperglycemic effect of the insulin is apparently not related to the ingestion of food. One rabbit, after a 60-hour fast, manifested a rise in the blood sugar of 35 mg.%, while in another instance, a well-fed rabbit which received food up to the time of the experiment, showed less hyperglycemia following the injection of insulin. In all instances where the hyperglycemia was transient, the maximal increase in blood sugar occurred at the 15-minute interval and disappeared within 60 minutes after the injection of insulin. No difference was observed in the blood sugar response to the massive doses of insulin when the analyses were made on blood obtained from the heart or from the marginal ear vein, although simultaneous studies on bloods obtained from these two sources were not carried out.

DISCUSSION

The fate of insulin injected intravenously is not clearly known. We have demonstrated that the amount of insulin recovered from the blood stream was greatest between 2 and 15 minutes after the injection. After 15 minutes, the amount present in the blood diminished rapidly. Our results show further that after 60 to 75 minutes, little or no insulin could be demonstrated in the blood stream by the method used. The insulin, however, must remain available to the tissues, since the depression of the blood sugar caused by insulin in amounts usually employed persists for at least 4 to 6 hours and with the larger doses used in this study for a much longer period of time.

Horsters (1) has shown that after the intravenous injections of smaller doses of insulin (20 U per kg. in rabbits), 10% of the insulin remained in the blood after 15 minutes and then increased in the organs. He further demonstrated that within 60 minutes, insulin was found in the muscle and remained there as long as it continued to act. After 210 minutes, the muscle contained 50% of the exogenous insulin. In our study, there was no appreciable difference between the insulin content of the 2 and 15-minute blood extracts, but there was a sharp loss of effect after 15 minutes. It was evident that the transfer of insulin from blood to organs and muscle became marked after 15 minutes and was relatively complete within 60 to 90 minutes.

The extracts made from blood obtained 90 to 180 minutes after the injection of the insulin caused a rise in blood sugar. The control blood extracts did not produce this rise. It is conceivable, therefore, that the increase in the blood sugar following the injection of these extracts may have been due to the liberation of some insulin antagonist or, as will be surmised later, due to the persistence in the blood of those factors responsible for the initial hyperglycemia after the insulin had been removed.

Blood sugar determinations on the rabbits receiving this massive dose of insulin demonstrated that for the first 60 minutes a hyperglycemic response was obtained. Of more interest is the fact that in 4 rabbits this persisted until the end of the experiment or until death of the animal. This paradoxical effect of insulin, i. e., the hyperglycemic response, was first observed by Rosello, Benatti and Balea (3) in a diabetic dog and by Guardabassi (4) in normal dogs; this problem was studied extensively (5, 6). These latter writers produced a marked hyperglycemia and glycosuria for as long as 4 hours in rabbits by repeated small intravenous injections of commercial insulin (2 to 5 U every 7 minutes). However, with the continued injection of insulin, the blood sugar finally decreased. These writers showed that glycogen was mobilized from the liver and as the liver glycogen became exhausted, the blood sugar fell. Analysis of the livers of their animals failed to reveal glycogen. Wichels and Lauber further demonstrated a more marked hyperglycemia in a well-fed rabbit than in a fasting one; however, the duration of the hyperglycemic effect was the same. Collens and Murlin (7), Bürger and Kramer (5) and Ionesco and his coworkers (8) showed that commercial insulin in contact with liver cells acted as an active glycogenolytic agent thus accounting for the initial hyperglycemia. Geiling and DeLawder (9) proved conclusively, however, that this initial hyperglycemia was due to impurities present in commercial insulin since it did not occur after the injection of the crystalline product. Subsequently, this observation was confirmed (10, 11, 12).

Although a rather highly purified insulin was used in the present experiments, it must be conceded that the minimal amount of impurities present in an average dose was exaggerated many-fold because of the large quantity of the product injected. The initial hyperglycemia noted in some of our animals may be explained on the basis of the glycogenolytic action of the impurities present. However, the marked persistent hyperglycemia in 4 rabbits lasting for 50 to 190 minutes after the injection

of a single large dose, as far as we know, has not been demonstrated before. We do not agree with other writers that the degree of insulin hyperglycemia is due entirely to the quantity of glycogen in the liver, since in 2 of our rabbits, one of which was fed until the time of the experiment and the other starved for 60 hours, no appreciable difference in the response of the blood sugar was observed. It seems more likely that some trigger mechanism is set into action which either acts directly on the glycogen mobilizing forces or stimulates the production of hormones antagonistic to insulin (13). Whatever the mechanism, excessive doses of insulin cause a derangement of carbohydrate metabolism since in one of the 2 rabbits which survived the 1000 U of insulin, acetone was present in the expired air 24 hours after the injection; the other rabbit was not tested. This phase of the problem will require further investigation.

It was observed that several of the rabbits died with convulsive seizures not unlike those seen in insulin shock even when the blood sugar was distinctly elevated. This may have been due to a marked difference between tissue and blood sugar. Recently, Kerr and Best (14), in an attempt to explain the difference in the hypoglycemic response at various blood sugar levels following the injection of protamine zinc insulin and of unmodified insulin, assumed that after unmodified insulin is injected, dextrose is rapidly used in the tissues and when insulin shock occurs, the tissue sugar is much lower than the blood sugar. It may be postulated, therefore, that the mobilization of glycogen stimulated by the massive doses of insulin, while elevating the blood sugar markedly, may have so depleted tissue sugar that convulsions simulating those seen in hypoglycemia ensued.

SUMMARY

Insulin injected intravenously in massive doses (1000 U) into rabbits was recovered from the blood stream for 60 to 90 minutes after the injection. After this time, insulin was not recovered from the blood by the procedure used, although the insulin effect as demonstrated by hypoglycemia persisted for as long as 9 hours. The largest recoveries of insulin were made within the first 15 minutes after injection. Blood extracts prepared after 75 minutes following the injection of massive doses of insulin, frequently gave a hyperglycemic response.

In 4 rabbits, this massive dose of insulin was followed by a gradual and persistent rise of the blood sugar; the usual hypoglycemic effect of insulin was not evident. These animals died in convulsive seizures not unlike those seen in insulin shock in spite of very high blood sugars. Five rabbits showed only a transient hyperglycemia lasting approximately one hour.

Evidence is presented to indicate that massive doses of insulin may so disturb carbohydrate metabolism as to result in ketosis.

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FURTHER STUDIES ON THE RELATION OF THE THYROID AND HYPOPHYSIS TO OVARIAN RESPONSES OF MENOPAUSE URINE EXTRACTS[1]

S. L. LEONARD AND J. DE FRATES

From the Bureau of Biological Research, Rutgers University and the Squibb Institute for Medical Research

NEW BRUNSWICK, NEW JERSEY

SINCE THE discovery that the thyroid secretion acts antagonistically to the follicle stimulating hormone (FSH) (1, 2), a report appeared in which it was stated that hypophysectomized female rats were more responsive to FSH than normal rats because of the hypofunction of thyroid which follows hypophysectomy (3). While studying the thyroid-hypophysis-sex relationship further, it was observed that certain extracts containing the follicle stimulating hormone did not always show the increased effectiveness in the absence of the thyroid and furthermore, other observations were at variance with those of Tyndale and Levine. In this report, we wish to present our results of studying the effect of certain menopause urine extracts, containing FSH, in thyroidectomized and hypophysectomized rats.

METHODS AND RESULTS

Two different extracts of menopause urine were used. One was prepared from the urine of one individual by a tannic acid precipitation method similar to that of Levine and Tyndale (4) and the other preparation was made by a modified alcoholic precipitation method produced from pooled samples of menopause urine from many individuals and designated as gamone. In either extract no attempt was made to separate the luteinizing hormone from the follicle stimulating hormone.

Hypophysectomized female rats. As a preliminary observation, 6 sexually immature female rats, 30 days old, were hypophysectomized and after 9 days were injected with 5, 10 and 20 mg. of gamone for 5 days. The ovaries at autopsy weighed from 53.0 to 82.6 mg. The ovaries of the injected rats contained large ripe follicles, a few corpora lutea when the largest dose was given, and stimulated interstitial cells. The typical estrous conditions prevailed in the uteri and vaginae.

In order to test the relative effectiveness of gamone in normal and hypophysectomized rats, doses of 1 to 1.5 mg. were injected into a series of rats for a period of 3 days. The injections were begun immediately or 5 days after the operation. Table 1 shows that the ovarian weights of the hypophysectomized animals ranged from 8.8 to 17.0 mg. (group average) and those of the normal females ranged from 20.0 to 46.7 mg. In no case in the 28 animals used were the stimulated ovaries of the hypophysectomized females heavier than those of the normals. Interstitial cell stimulation was observed in the ovaries of the hypophysectomized rats but ripe follicles and corpora lutea were found in addition only in the ovaries of the unoperated injected animals. The uterine weights of the hypophysectomized rats were also lighter than those of the normal rats.

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TABLE 1. EFFECT OF HYPOPHYSECTOMY ON THE RESPONSE OF MENOPAUSE URINE EXTRACTS

No. of rats	No. days after hypophysectomy	Treatment	Av. ovarian wt.		Av. uterine wt.		Difference	
			Exp.	Control	Exp.	Control	Ovary	Uterus
			mg.	mg.	mg.	mg.	%	%
5	0	1 mg. gamone	13.0	34.2	138	176	−62	−22
6	0	1.5 mg. gamone	17.0	26.3	—	—	−35	—
2	5	1 mg. gamone	13.0	46.0	61	160	−72	−62
4	5	1.5 mg. gamone	12.7	46.7	49	247	−73	−80
2	5	1.5 mg. gamone	8.8	20.0	22	136	−56	−84
3	5	1.5 mg. gamone	17.0	35.0	26	131	−51	−80
6	5	1.5 mg. gamone	13.0	35.0	35	192	−61	−81
2	0	40 mg. M.U.	18.7	49.2	101	140	−62	−28
2	0	45 mg. M.U.	26.5	28.3	117	123	− 6	− 5
9	1	50 mg. M.U.	24.3	38.8	106	132	−40	−20

These results were not expected, particularly, since Tyndale and Levine (3) showed that hypophysectomized rat ovaries were more sensitive than normal rat ovaries to the follicle stimulating hormone from menopause urine. In view of these findings, a tannic acid extract of menopause urine from one individual was injected into another group of rats under the same conditions (13 animals). The ovaries of the hypophysectomized rats weighed from 16.5 to 26.5 mg. and those of the controls weighed from 28.3 to 49.2 mg. (table 1). In no instance did the hypophysectomized rats show a greater response than the normals. Only follicular stimulation was produced in the ovaries of the operated rats while the normal rats had follicles and in some animals a few corpora lutea in their ovaries. Except for the fact that this extract produced only follicles in the hypophysectomized rats' ovaries at the doses given, the results were similar to those obtained with gamone.

Thyroidectomized female rats. In the next experiment an attempt was made to repeat an earlier observation (2) to determine if these particular extracts were more effective in the thyroidectomized female rat than in the normals. Immature female rats were thyroidectomized and after several days were injected with similar doses of gamone or with the same tannic acid preparation used previously. Table 2 shows that the removal of the thyroid had no effect on the ovarian response to gamone but that the other menopause urine extract was more effective in the thyroidectomized rats. In the latter case, the greater uterine weight reflected the greater ovarian follicular stimulation in the thyroidectomized rats.

Hypophysectomized male rats. In view of the fact that gamone gave interstitial cell stimulation in the ovaries, it seemed likely that the extract contained some luteinizing hormone. To test this, hypophysectomized male rats were injected with gamone and the effects on the testes and accessory reproductive organs were noted. According to Smith and Engle (5) and Greep, Fevold and Hisaw (6), the FSH produces only

TABLE 2. EFFECT OF THYROIDECTOMY ON THE RESPONSE OF MENOPAUSE URINE EXTRACTS

No. of rats	Treatment[1]	Av. ovarian wt.		Av. uterine wt.		Difference	
		Exp.	Control	Exp.	Control	Ovary	Uterus
		mg.	mg.	mg.	mg.	%	%
6	1.5 mg. gamone	32.1	29.5	132	131	9	0
5	1.5 mg. gamone	24.8	25.9	131	130	4	0
8	34 mg. M.U. extract	35.1	24.9	127	77	41	65

[1] Injections made over a period of 6 days.

sperm in the tubules and that any luteinizing substance present will stimulate the interstitial cells with a resulting increase in the accessory organs of reproduction. Seven young adult male rats were hypophysectomized and after a lapse of 7 to 10 days were injected with a total dose of 8 to 30 mg. of gamone for a period of 10 days. Four rats served as controls. The testes and accessory organs of the hypophysectomized injected males weighed, as an average, 1.260 and 2.192 gm. respectively, while the controls weighed 0.690 and 0.645 respectively. Spermatids were found in all the stimulated testes and fully formed sperm in 5 of the 7 rats while the controls showed the usual degenerative changes. The interstitial cells of the testes were greatly stimulated and the accessory organs of reproduction approached in size to those of a normal rat of the same age.

DISCUSSION

It is noted that gamone was not more effective in increasing the ovarian weight in either the hypophysectomized or thyroidectomized female rat but that the menopause urine extract from one indvidual was more effective in the thyroidectomized rat alone. There is no doubt that gamone contains both follicle stimulating and luteinizing substances when tested in relatively large doses in hypophysectomized male and female rats. The other extract contained a larger amount of FSH because only follicles were produced in the hypophysectomized rats at the doses given and there was no evidence of interstitial cell stimulation. Apparently the presence of luteinizing hormone interferes with the increased ovarian response to FSH in the thyroidectomized animal.

On a theoretical basis this does not seem as it should be. If, when a mixture of FSH and luteinizing hormone (LH) are used to increase the size of an ovary, one augments the other in the result produced (7); to increase the FSH would further increase the size of the ovaries by augmentation. Does it not seem logical to expect that the increase in the effectiveness of the FSH obtained by thyroidectomy would be sufficient to give an augmented ovary in the presence of both FSH and LH? The interrelationship is more complex than it first appears.

It is impossible at present to reconcile the results obtained by Tyndale and Levine (3) with those secured here in the hypophysectomized female rat, Since gamone was not more effective in the thyroidectomized rat, it might not be expected to be more effective in the hypophysectomized rat. Tyndale and Levine reported that if the dosage of their hormone was sufficient to produce large luteinized ovaries in their control animals, then the ovaries of the treated hypophysectomized animals, were not larger than their treated controls. In our experience, however, the tannic acid extract produced ovarian weights and follicular stimulation similar to those obtained by Tyndale and Levine and yet the results did not confirm theirs. Furthermore, when the injections were started on the same or one day following hypophysectomy, the ovarian weights of the operated rats were not even equal to their controls but invariably less. Neither were the uterine weights of the hypophysectomized injected rats as large as those of the injected control animals.

There is a possibility that the individual (or individuals?) from which Tyndale and Levine obtained their FSH might have been a much purer source of FSH than those reported here, or that their preparation may have been more highly purified for FSH than either gamone or our tannic acid extract. The latter possibility does not seem likely because there was no indication of separating the LH from their extract in the method of preparation. If the first possibility is true, then there must be a wide variation in the FSH and LH content in menopause urine.

SUMMARY

Two menopause urine extracts, one prepared from pooled samples from many individuals (gamone), another prepared by the tannic acid precipitation method, from one individual were injected into normal, hypophysectomized and thyroidectomized immature female rats. Neither extract was as effective on the ovaries of the hypophysectomized rat as in the controls. In the thyroidectomized rats, the extract of urine from one individual was more effective than in the control, but this was not true for gamone. Gamone was found to be quite rich in luteinizing hormone when tested on hypophysectomized male rats. This, perhaps, was the cause of the failure to induce a greater ovarian response in the thyroidectomized rats than in the controls. We were unable to observe that the hypophysectomized female rat was more responsive to preparations from menopause urine containing the follicle stimulating hormone.

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THE MODE OF ACTION OF TESTOSTERONE PROPIONATE ON THE FEMALE GENITAL TRACT

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IT HAS BEEN demonstrated that menstruation and ovulation are inhibited in monkeys (1, 2) by the administration of testosterone propionate and that its injection into laboratory animals prevents follicular and luteinizing changes in the ovary (12). Browman (3) reported the suppression of the vaginal estrous cycle in rats by injections of testosterone propionate, a finding Papanicolaou (4) has been able to duplicate in women after androgenic therapy. On the basis of these laboratory and clinical investigations the use of testosterone propionate for certain gynecologic conditions, especially functional uterine bleeding, has been advocated and reports (5, 6, 10, 11) as to its value have appeared in the literature. Geist and his coworkers rapidly controlled functional bleeding in 23 of 25 women treated with testosterone propionate in dosages varying from 300 to 1000 mg. per month. They found that regression of the endometrium to a state of hypoplasia or atrophy occurred when large amounts were given and that, following discontinuance of the therapy, the inhibitory phenomena gradually disappeared and normal estrogen and progestin effects recurred. They noted a moderate increase in facial hirsutes in 4 patients who had, however, definite hirsutes before treatment was instituted and 3 patients in whom slight coarsening of the voice developed. Most of these adverse symptoms disappeared on cessation of treatment. Mazer and Mazer (14) using approximately the same dosages have recently reported similar results with no notable ill effects from the treatment recorded.

During the past year one of us (J.W.H.) has had occasion to use testosterone propionate[1] for the control of functional uterine bleeding in a group of patients and has been able to confirm the findings of Geist and others. Ten women, a number insufficient for critical analysis but adequate for corroborative purposes, have been followed for a time long enough to warrant reporting. In this group severe functional bleeding and normal menstrual flow were inhibited for periods up to 86 days by intramuscular injections of 25 mg. of testosterone propionate given 3 times a week for 6 weeks. Bleeding did not stop entirely until 10 or more injections had been administered. Doses of 10 to 25 mg. of this androgen, given twice weekly to patients with prolonged or unusually heavy menses, shortened the periods and decreased the flow but did not prevent the onset of menstruation.

In this small group of women no generalized masculinizing effects were noted, although one patient thought that perhaps the facial hair, which she possessed before treatment, became somewhat darker, and 2 others, who received the largest

[1] Testosterone propionate in the form of perandren was very kindly furnished for this investigation by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

dosage, had definite clitoridal enlargement which subsided on cessation of treatment. Most of the patients gained weight. Six reported a sense of unusual well-being. None reported a decrease in libido and 2 voluntarily stated that their interest in sex-life had apparently increased somewhat after treatment was begun.

Effect of Testosterone Propionate on the Ovarian Response to Gonadotropic Substance

The use of a potent endocrine substance in amounts capable of producing the changes ascribed to testosterone suggested the desirability of making an intensive study of its mode of action. It has been suggested by several investigators (7, 8) that the production of changes in the female genital tract by administration of testosterone propionate may be due to inhibition of the pituitary gland. However, a direct inhibitory effect on the ovary as a result of androgenic action has never been clearly excluded, although Robeson's investigations (9) are suggestive. It seems worth while, therefore, to report the effect of injections of testosterone propionate on the reaction of the rabbit ovary to gonadotropic material.

Method. Isolated adult virgin female rabbits weighing 2.5 kg. or more were used throughout the experiment. Extract of pregnant mare serum[2] was utilized as a gonadotropic substance because of its known ability to produce ovulation. Testosterone propionate was the androgen administered.

Initial intravenous injections of the gonadotropic material in amounts, 2 to 5 Upjohn rat units (13), sufficient to cause definite ovarian changes were given. Twenty-four to 36 hours later the rabbits were subjected to laparotomy under ether anesthesia. The genitalia were examined for evidence of stimulation and one ovary and a cornu of the uterus were removed from each animal for controls. The animals were kept in clean, well aired and well lighted quarters and on an adequate diet.

Three to 5 days after operation each rabbit was given an intramuscular injection of 2.5 mg. of testosterone propionate and similar injections were made at intervals of every other day for at least 30 days. Gonadotropic substance in the same amount as used in the initial injection was again administered intravenously on the same day the last dose of testosterone was given. Twenty-four to 36 hours later the animal was killed and the genitalia removed.

The ovaries and uterine cornua were sectioned. Histologic studies were made for evidence of gonadotropic reaction occurring in the genitalia.

Some of the animals received smaller amounts of gonadotropic material than others so that what might be termed minimal effects could be observed. The time of observation following injection of gonadogen was kept within a 36-hour period in order to obtain, if possible, histologic evidence of recent ovulation (fig. 1, B).

Fifteen animals were used, of which four were controls.

RESULTS

These are summarized in table 1. In every instance where a response in the ovary (as indicated grossly by corpora hemorrhagica and microscopically by evidences of recent ovulation, early corpora lutea or greatly increased numbers of large follicles) occurred following the initial injection of the pituitary-like substance, the same size dose when repeated produced comparable changes in the remaining ovary despite prolonged administration of testosterone propionate (fig. 1, D and E). The uteri

[2] Extract of pregnant mare serum in the form of gonadogen was very kindly furnished by the Upjohn Company, Kalamazoo, Michigan.

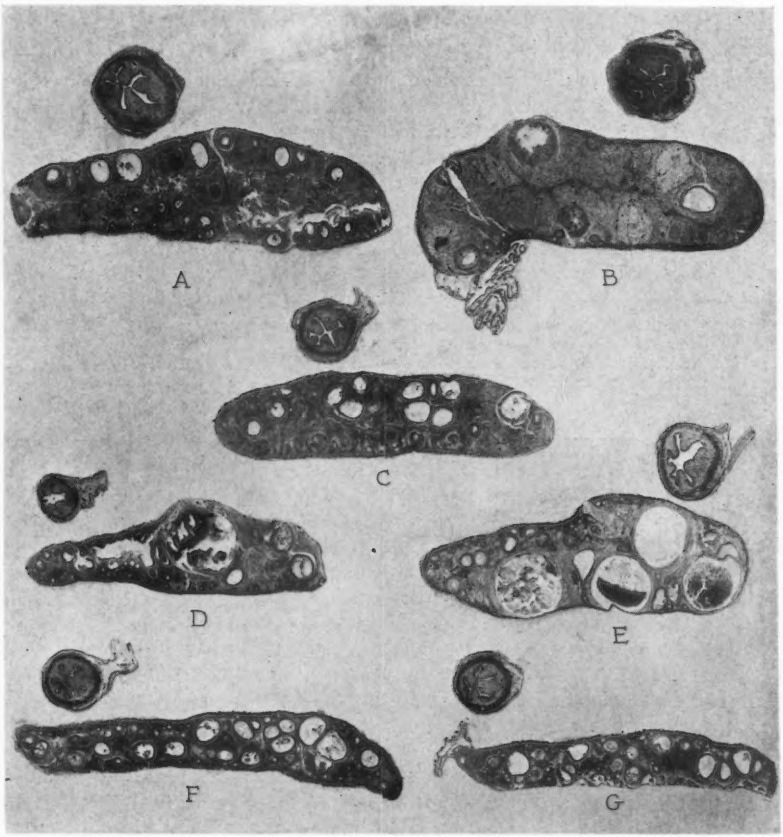

Fig. 1. PHOTOMICROGRAPHS OF OVARIES AND UTERINE CORNUA FROM ISOLATED ADULT VIRGIN RABBITS, (×5.5). A. Normal. B. Thirty-six hours after the intravenous injection of 2 Upjohn rat units of gonadogen (pregnant mare serum) showing stoma of recent ovulation. C. Appearance after 18 injections, one given every other day, of 2.5 mg. of testosterone propionate. Gonadogen, 3 U.R.U. (sufficient to produce a marked gonadotropic response) had been administered prior to instituting the injections of testosterone. Shown to demonstrate the absence of any gonadotropic stimulation remaining from the original injection of gonadotropic material given 35 days previously. D. Right ovary and uterine cornu 36 hours after injection of 3 U.R.U. of gonadogen. Gonadotropic response evidenced by corpora hemorrhagica. E. Left ovary and uterine cornu of same animal as in D, 18 injections of testosterone propionate, each 2.5 mg., one given every other day, were followed by the administration of 3 U.R.U. of pregnant mare serum. Gonadotropic response shown by corpora hemorrhagica. F. Right ovary and uterine cornu, normal. G. Left ovary and uterine cornu, from same animal as in F, removed following 18 injections of 2.5 mg. of testosterone propionate, one given every other day.

responded somewhat, grossly, to the gonadotropic material but there was no histological evidence that they were affected by the injections (fig. 1).

It will be noted that 2 rabbits received testosterone propionate without the administration of any gonadotropic substance, and that the ovaries and uteri were apparently unaffected by the procedure (fig. 1, F and G); two other animals had no initial injection of gonadogen and the response in their ovaries to 3 R.U. of pregnant mare serum, after prolonged administration of testosterone propionate, was essentially a normal one.

DISCUSSION

The value of a substance capable of stopping serious functional uterine bleeding in women is inestimable. When, however, that substance is a potent androgen which not only inhibits functional bleeding but also affects the female genital tract so that all cyclic phenomena cease, then its widespread gynecologic use should be recommended only after sufficient experimental evidence has been produced to indicate its mode of action and its probable ultimate effect upon the individual. It would appear from a review of the available case reports now present in the literature (April, 1939) that there will be instances in which some degree of masculinity will develop

TABLE 1

Rabbit No.	Initial dose of gonadogen in Upjohn R.U.	Appearance of genitalia at initial examination	No. of inject. of test. prop. (2.5 mg. each)	Final dose of gonadogen in R.U.	Appearance of genitalia at final examination
1, 2	5	corpora hemorrhagica early corpora lutea uterine hypertrophy and hyperemia	17	5	corpora hemorrhagica recent ovulation uterine hypertrophy and hyperemia
13	4	corpora hemorrhagica early corpus luteum uterine hyperemia	18	4	corpora hemorrhagica recent ovulation uterine hyperemia and hypertrophy
5, 6, 7	3	corpora hemorrhagica recent ovulation early corpora lutea uterine hypertrophy and/or hyperemia	17	3	corpora hemorrhagica recent ovulation early corpora lutea uteri small, hyperemic
11, 12	3	recent ovulation early corpora lutea uterine hyperemia and/or hypertrophy	18	none	occasional small follicles without evidence of activity uteri small, atrophic
10, 9	2	follicular activity corpora hemorrhagica (1) uteri hyperemic endometrial hypertrophy in *rabbit* 9	16	2	follicular activity corpora hemorrhagica uterine hypertrophy no endometrial hypertrophy
3	1	follicular activity no ovulation uterus small pale	17	none	no follicular activity ovary small, pale uterus atrophic
8, 4	none	no ovarian activity uterus small pale	17	3	corpora hemorrhagica follicular activity uterus hyperemic, hypertrophied
14, 15	none	no ovarian activity uterus small, pink	18	none	small follicles, no ovarian activity, uteri, small, pale

after the administration of testosterone propionate. Proper control of the dosage and careful observation of the patient should reduce such untoward results to a minimum. We offer the suggestion that testosterone propionate accentuates only those masculine characteristics which are already present in the woman affected. If such is the case it would appear advisable to use some means other than androgenic therapy in the treatment of functional bleeding in women who have an appreciable degree of facial or pectoral hirsutes, low pitched voices, large clitoridal organs or other definite android characteristics.

As previously mentioned, several authors have advanced the hypothesis that the administration of androgens inhibits pituitary activity. Hamilton and Wolfe (15)

have demonstrated that the injection of a synthetic androgen will reduce, in part, the gonadotropic properties of the rat pituitary. This they have shown by transplanting the hypophysis of androgen-injected rats into immature females of the same species and comparing the ovarian weights of the latter with the ovarian weights of controls.

The ovarian cycle is a response to the gonadotropic activity of the hypophysis. In the rabbit this response occurs normally only after coitus but can be produced artificially by the administration of gonadotropic material. It is to be expected that the ovary, if it is affected directly by an androgen, will not respond to the same amount of a pituitary-like substance after injections of testosterone propionate as before the injections were given. If, however, the effect of the androgen is primarily on the pituitary and not on the ovary, then the latter should respond to gonadotropic material given intravenously even though the hypophysis is inactive. Our results appear to validate this reasoning.

SUMMARY

We have been able, in a group of patients, to corroborate the findings of others that testosterone propionate will inhibit functional uterine bleeding and menstruation in women when given in adequate dosage. Manipulation of the treatment can roughly control the duration and the amount of the menstrual flow. Such a potent substance as testosterone propionate requires intensive study and we have endeavored to examine, by laboratory experiments, its mode of action on the female genital tract. This contribution is concerned with the first step in this procedure—a determination of what effect, if any, testosterone propionate has upon the reaction of the ovary to gonadotropic material. It was found that the rabbit ovary is stimulated by extract of pregnant mare serum despite the previous administration of testosterone propionate. This observation, when correlated with the findings of Hamilton and Wolfe that testosterone decreases the gonadotropic effect of the pituitary, will adequately explain the modus operandi of this androgen in inhibiting the cyclic phenomena of the female genital tract.

Previous reports to the effect that testosterone propionate is capable of inhibiting functional uterine bleeding and menstruation in women were corroborated.

Pregnant mare serum extract produced similar responses in the rabbit ovary before and after prolonged administration of testosterone propionate. It would appear that the action of testosterone propionate in inhibiting the cyclic changes in the female genital tract is not a direct one upon the ovary; rather it would appear to be a result of pituitary inhibition.

The authors wish to express their appreciation to Dr. Arthur H. Curtis for his helpful counsel during the course of this investigation.

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OBESITY IN THE RAT FOLLOWING THE INJECTION OF CHROMIC ACID INTO THE HYPOPHYSIS[1]

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OBESITY HAS BEEN produced experimentally in animals by a number of different workers using various procedures directed at the structures located at the base of the brain. The following observations have been made in dogs: Crowe, Cushing and Homans (1, 2) observed adiposity in animals partially hypophysectomized; and their conclusions were supported by Dott (3), who also used such technics as stalk section and placing of a silver clip to show that (in his opinion) circulatory disturbances of the anterior lobe gave the same net result. Clip insertion was also claimed to have produced several cases of obesity and infantilism (4, 5); the first of these did not confirm Cushing and his coworkers in their findings regarding partial hypophysectomy, but Dandy and Reichert (6) were in substantial agreement with Crowe, Cushing and Homans. Aschner (7) observed obesity in both immature and adult specimens, but much more markedly in the former, after complete hypophysectomy. It has been produced by damaging the hypothalamus in the neighborhood of the hypophysis (8–11); Curtis (12) did not concur in these findings, and all other workers cited had many negative as well as positive cases.

After the dog, the animal most used in experiments dealing with this field has been the rat. Work on this species was begun by Smith, Walker and Graeser (13), but a paper published by Smith (14) appears to represent the final views of this investigator. He reported that he had injected chromic acid into the hypophyses of rats and produced adiposity; but because outright removal of the entire hypophysis did not bring about the same result, he surmised that concomitant damage to the hypothalamus was responsible for the outcome. He did not detail the damage sustained by the hypophysis in his cases, nor the suspected injury to the diencephalon. He stated, however, that contrary to his findings in young animals, rats completely hypophysectomized at about the time of sexual maturity sometimes showed moderate fat deposition. In addition Smith (15) reported a case of adiposity after partial removal of the anterior lobe; this single example agrees with the work of Reiss, Epstein and Gothe (16), who, however, reported it as a constant phenomenon in their series. Crooke and Gilmour (17) found hypothalamic damage in all their obese rats, but had even more negative cases with similar hypothalamic involvement. Hetherington and Ranson (18) have recently published a preliminary report indicating adiposity was a practically constant result in young rats in which electrolytic lesions were placed in the hypothalamus with the Horsley-Clarke instrument.

Insofar as other species are concerned, about the only experiments dealing with the subject are those summarized by Ranson, Fisher and Ingram (19). They report the case of one monkey in which hypothalamic lesions had been placed, and which

[1] Aided by a grant from the Rockefeller Foundation.

showed marked obesity after a short time. Because of various considerations, and on the basis of a long series of experiments on monkeys and cats (and more recently guinea pigs) these workers stated their belief that proof for hypothalamic regulation of fat metabolism was lacking.

As this brief review brings out, the main question at issue concerning the cause of adiposity related to structures in the region of the base of the brain has been whether the hypophysis or the hypothalamus plays the rôle of major importance in the etiology of such disorders. Most experiments designed to distinguish between the parts played by these two portions in fat metabolism have run afoul the difficulty inherent in their close anatomical relationship. Those reported here were inaugurated with the object of checking the work of Smith (14).

METHODS

Operations were performed via the parapharyngeal approach to the hypophysis originally devised by Smith, but in approximately the form suggested by Richter (20). Evipal (0.1 gm. per kg. of body weight) was used for anesthesia. After the pituitary gland was exposed a small amount (usually 0.01 cc.) of a 5% aqueous solution of chromic acid was injected into the hypophysis. For this purpose a 1-inch 27-gauge needle with its tip bent 2 mm. back from the point to form about a 100° angle with the rest of the needle, was used. This was fastened to a tuberculin syringe held tightly in a light metal frame with a screw and a spring so attached to the plunger that it could be pressed down slowly and an accurately measured amount of the acid expelled. The actual injection of the acid into the hypophysis was made as follows: The operator lowered the bent tip of the needle through the opening in the sphenoid bone and inserted the point forward into the hypophysis, supporting the whole apparatus; while an assistant at a signal turned the screw at the top of the frame, forcing the acid into the hypophyseal tissue. This was, of course, all done with a minimum of movement of the gland.

All operated animals had litter-mate controls and were allowed to survive for at least 3 months. They were weighed at weekly intervals and were measured for length at the beginning and end of the experimental period. At autopsy the brains were removed and fixed in formalin. The fresh hypophyses were detached and fixed in either Zenker-formol fixative or Champy's fluid. Glands fixed in the former were stained by a modification of the Haterius technic; those in the latter by the Severinghaus method. The proportion of the hypophysis remaining was estimated by the method of Rasmussen and Herrick (21). The brains were sectioned serially and stained with cresyl violet. The other endocrine glands were sectioned at 8 micra and stained by Van Gieson's method.

After the brain was removed at autopsy from 2 of the obese rats and their controls, the bodies were skinned and run through a meat chopper. The fat was then extracted from this material with both acetone and alcohol [see Hetherington and Weil (22), and work to be published].

RESULTS

A total of 51 animals survived the operation and lived until they were killed for autopsy. Many showed no disturbances during life, and it was discovered at autopsy that no perceptible damage to the hypophysis or hypothalamus was evident. Twenty-two rats were studied in considerable detail because they showed striking symptoms after the operation. Data on these animals are summarized in the table.

Of the entire series, however, only 3 displayed any noticeable degree of adiposity. This put in its appearance in from 6 to 8 weeks after the operation. All 3 of the fat

TABLE 1. DATA ON OPERATED ANIMALS AND THEIR CONTROLS

Rat No.	Op. or cont.	Time of operation			Time of autopsy			Proportion of hypophysis left			Hypothalamic damage
		Age	Weight	Length	Age	Weight	Length	Ant.	Int.	Neur.	
		days	gm.	mm.	days	gm.	mm.	%	%	%	
Ra- 7	O	53	80	285	252	285	414	95	57	100	None
Ra- 8	O	53	100	312	330	304	381	L.R.H.[1]	L.R.H.	L.R.H.	Extensive[2]
Ra- 9	C	53	95	299	330	344	421	100	100	100	—
Ra-11	O	56	95	305	333	343	362	24	39	36	Extensive[2]
Ra-12	O	56	80	297	252	180	360	49	32	36	None
Ra-13	C	56	85	296	333	245	390	100	100	100	—
Ra-18	O	56	75	298	166	220	362	37	33	56	None
Ra-21	C	56	65	296	166	250	390	100	100	100	—
Ra-31	O	39	65	248	146	170	354	44	14	25	Slight[2]
Ra-32	C	39	65	250	146	170	357	100	100	100	—
Ra-33	O	42	75	258	172	145	332	41	21	29	None
Ra-34	C	42	75	265	172	180	363	100	100	100	—
Ra-35	O	47	95	293	171	270	407	32	+[3]	+	Slight[2]
Ra-36	C	47	100	283	171	350	422	100	100	100	—
Ra-37	O	47	80	263	172	175	356	44	28	42	None
Ra-38	C	47	85	282	172	205	380	100	100	100	—
Ra-39	O	51	90	292	152	170	366	67	88	70	None
Ra-40	C	51	75	275	152	185	366	100	100	100	—
Ra-41	O	49	80	282	146	285	407	65	71	51	None
Ra-42	C	49	80	282	146	305	425	100	100	100	—
Ra-43	O	53	135	326	168	200	373	20	19	33	Slight[2]
Ra-45	O	53	115	311	248	277	366	26	67	100	Extensive[2]
Ra-46	C	53	120	321	248	260	403	100	100	100	—
Ra-53	O	59	145	338	146	165	376	18	25	21	Slight[2]
Ra-54	C	59	115	319	146	320	430	100	100	100	—
Ra-57	O	45	100	307	135	216	377	11	23	65	Slight[2]
Ra-58	C	45	90	296	135	306	445	100	100	100	—
Ra-59	O	50	105	300	135	132	317	L.R.H.	L.R.H.	L.R.H.	None
Ra-60	C	50	90	292	135	200	380	100	100	100	—
Ra-61	O	54	100	307	143	155	335	L.R.H.	L.R.H.	L.R.H.	Slight[2]
Ra-62	C	54	90	300	143	306	408	100	100	100	—
Ra-63	O	53	110	305	118	267	405	57	31	80	None
Ra-64	O	53	100	312	118	255	411	48	15	22	None
Ra-65	C	53	120	318	118	267	396	100	100	100	—

[1] L.R.H., Little recognizable hypophyseal tissue. Much scar tissue.
[2] *Ra-8*, *Ra-11* and *Ra-45* were obese animals. See comment in text.
+ [3] Parts of intermediate and neural lobes found with hypothalamus.

rats were dwarfed in comparison to their controls, but in spite of this fact 2 (*Ra-11* and *Ra-45*) actually exceeded the latter in weight. Quantitative determinations of the total fat in these 2 showed that the carcass of *Ra-11* contained almost 50% by weight of lipoids compared to about 26% for its control; while *Ra-45* contained about 37% compared to about 21% for the control.

Hypothalamic damage. Microscopic examination of serial sections of the brains

of these fat animals revealed very extensive and essentially similar damage to the hypothalamus and the medial edge of the pyriform lobe in all 3. This damage took the form of softening and cell loss for the most part bilateral in extent affecting principally the superficial parts of the base of the brain in those areas. The tuberculum olfactorium, the supraoptic nucleus, the nucleus ovoideus, the optic chiasma and the median eminence were more or less affected in all 3 rats. In *Ra-8* and *Ra-11* the ventral edge of the anterior hypothalamic area showed some softening. *Ra-8* and *Ra-45* displayed involvement of the ventral edge of the ventromedial hypothalamic nucleus and of the mammillary body; and *Ra-11* and especially *Ra-45* showed some destruction of the lateral hypothalamic area. The damage was usually not symmetrical in any animal, being more pronounced on one side or the other.

With respect to the remaining operated animals whose brains were examined histologically, it may be said that not one showed more than a small fraction of the destruction evident in the obese rats. In most no damage whatever was visible. In several rats there was a small amount of softening located at some point or other in the ventral part of the hypothalamus, and a few displayed such features as thickening of the pia-arachnoid membrane, some gliosis of the optic chiasma and thinning of the median eminence.

Hypophyseal damage. Changes were apparent in the anterior lobes of the hypophyses of all 3 fat animals. In *Ra-8* nothing but a few nests of deeply staining basophilic cells could be found here and there in the sella. In *Ra-11*, in which adiposity was most marked, a considerable portion of the anterior lobe, which was about two-thirds destroyed, was occupied by a scattered branching network of basophilic cells resembling an adenoma. *Ra-45* which also had about two-thirds to three-quarters of the gland destroyed, showed an apparent degranulation of many of the basophils. Both of these last 2 cases exhibited as well an abnormality of the acidophils in that the cells were both smaller and less numerous than usual. These same glands had nearly the normal portions of pars intermedia and pars nervosa.

Eighteen hypophyses taken from non-obese examples displayed a wide range of destruction from practically complete elimination of the gland to very little disturbance. In most cases these anterior lobe fragments, regardless of the size of the piece concerned, presented an essentially normal cytological picture after differential staining.

Changes in other organs. The testes, adrenals and thyroid (as were also the pancreatic islets and liver) of *Ra-8* were practically normal, though a little smaller than those of the control; *Ra-8* was considerably smaller than its litter mate. *Ra-11* displayed a somewhat flattened thyroid epithelium and small ovaries containing no corpora lutea. The cells of the cortical zone of the adrenal glands contained more vacuoles than usual, perhaps from the accumulation of fat; this was true also of the liver. The pancreatic islets were normal. The organs of *Ra-45* showed no abnormalities save for some increase in vacuoles in the liver cells and possibly a slight increase in interfollicular epithelial cells (not well organized) in the thyroid.

DISCUSSION

As may readily be understood from the foregoing our experiments might have had a completely negative outcome except for several fortunate operative accidents. Thus, in a number of cases where the hypophysis was destroyed in various proportions by the injection of chromic acid no excessive fat deposition was obtained. Even minor damage to the hypothalamus accompanying the hypophyseal procedure appeared to be insufficient to cause adiposity. But when the operation on the hypophysis resulted in extensive injury to the hypothalamus obesity was produced. We have

had no examples of adiposity to date in which it has been possible to prove the pituitary gland was intact, though experiments now in progress may throw light on this aspect of the matter later.

The striking though seemingly inconsistent cytological picture found in the hypophyses of the 3 fat animals brings up the question, as so many of the recent studies do (23–26), of what part the cells of the hypophysis, particularly the basophils, may play in fat metabolism.

Although the evidence is still too limited to make any certain forecast of what the final solution of the problem of the relationship to obesity of lesions at the base of the brain may be, we are inclined at present to the belief that in the rat at least both the hypophysis and the hypothalamus may be involved. Whether they will turn out to be both concerned but independent of each other, or mutually interdependent with perhaps one taking a primary rôle we cannot venture to predict. At any rate we are of the opinion that insofar as the rat is concerned we must credit the hypothalamus with great importance in the causation of obesity.

SUMMARY

Experiments are described in which chromic acid was injected into the hypophyses of rats via a parapharyngeal approach. Only 3 of 51 animals so operated showed any resultant adiposity. Chemical analysis showed these animals contained up to 50% more fat than their litter-mate controls. All were somewhat dwarfed. Histological examination revealed that extensive damage to the hypothalami and hypophyses of the fat rats had been done; while among the negative cases hypothalamic injury was minor or absent, although hypophyseal destruction was often severe. No obese cases with intact hypophyses were discovered. The hypophyses of the fat rats varied in the histological picture they displayed, as did some of the other endocrine glands.

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ANDROGENIC ACTION OF DESOXYCORTICOSTERONE[1]

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IT IS BECOMING increasingly evident that under certain conditions the adrenal cortex exerts androgenic action. That various degrees of masculinization accompany adrenal cortical tumors in human females is well known. In the castrated rat Davidson (1) has reported stimulation of the prostate and seminal vesicles by the administration of adrenotropic hormone. In the immature mouse Howard (2) has found that while castration resulted in none of the histologic changes in the prostate and seminal vesicles which are produced by castration of the adult mouse, such changes were produced by castration and adrenalectomy of animals kept alive by a high salt intake.

The means by which the androgenic effects of the adrenal are mediated is not as yet entirely clear. There is often high androgenic activity in the urine of patients with virilism and adrenal tumors (3–6), but this finding is inconstant (3, 6). Extracts of adrenal glands appear to be inactive as androgens (7), and apparently none of the testicular or urinary androgens has been isolated from adrenal tissue. Richstein (8), however, has obtained from adrenal glands a compound, adrenosterone, which proved to be one-fifth as active as androsterone in comb-growth stimulating power, but was inactive in maintaining adrenalectomized animals.

It is just possible that the essential hormones of the adrenal cortex in the pure state may possess androgenic properties. Among the compounds which have been obtained from cortical tissue, desoxycorticosterone (9) has been shown to prolong the survival of adrenalectomized animals (10) and to be effective in Addison's disease (11), and may therefore be considered a true adrenal cortical hormone. Accordingly, we have subjected this compound to the accepted biologic tests for androgenic activity.

PROCEDURE AND RESULTS

Synthetic desoxycorticosterone acetate dissolved in sesame oil in a concentration of 5 mg. per cc. was the material employed, and its ability to stimulate comb growth and to prevent castration changes in the prostate and seminal vesicles of mice and rats has been examined.

Comb growth. As a preliminary test a single White Leghorn capon was given 4 daily subcutaneous injections of 2.5 mg. of desoxycorticosterone acetate. On the fifth day the comb had increased 6.5 mm. in size (length plus height). A second capon was given 5 daily injections of 1.5 mg. of the hormone, the response to which was an increase in comb size of 1.5 mm. Four capons were then given 5 daily injections of 2.0 mg. with resulting comb growth responses of 3.0, 3.0, 3.5 and 3.0 mm. The compound thus had a definite but rather low comb-growth stimulating action. As a first

[1] This study was aided by a grant from the Fluid Research Funds of Yale University School of Medicine. The desoxycorticosterone acetate used was generously supplied by Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc.

approximation, it seems that, measured by this standard, desoxycorticosterone acetate possesses androgenic activity to the extent of slightly more than 3.0 Gallagher-Koch bird units (12) in 10 mg.; or, 1 mg. has approximately 0.3 B.U. activity as an androgen. Thus the compound was approximately one thirty-third as active as androsterone in stimulating comb growth.

The response of the comb differed in no evident way from the response to small quantities of male hormone substances. Upon the termination of injections the combs promptly regressed and regained their pre-treatment sizes.

Mice. Six young adult mice were castrated. Two of these mice received 0.5 mg. and 2 mice received 0.25 mg. of desoxycorticosterone acetate subcutaneously every second day, beginning the day after castration. The remaining 2 mice were kept as untreated controls. After 11 days the animals were killed. As compared with the untreated castrates, the animals given the higher dose showed appreciable stimulation grossly of both the prostate and the seminal vesicles; the animals receiving the smaller dose showed some prostatic stimulation, but the seminal vesicles were stimulated very slightly if at all. It has been our experience that the mouse prostate and

TABLE 1

Rat No.	Treatment	Organ weights		
		Seminal vesicles	Adrenals	Hypophysis
		mg.	mg.	mg.
1	None	149	56	10
2	1 mg. DC[1] daily	248	54	12
3	1 mg. DC daily	178	46	11
4	10 γ testosterone propionate daily	209	41	11
5	5 γ testosterone propionate daily	231	41	11

[1] Desoxycorticosterone acetate.

seminal vesicles show a very indefinite and uncertain histologic response to amounts of androgen minimal for weight stimulation. For this reason it is difficult to be sure whether there was significant histologic stimulation by desoxycorticosterone; what response there may have been was slight.

Rats. Five male rats 6 months of age were castrated. Beginning the day after operation 2 of the rats were given daily injections of 1.0 mg. of desoxycorticosterone acetate. For comparison, one rat was given 5γ and one rat 10γ of testosterone propionate daily, while the remaining animal was reserved as an untreated castrate. Ten days later the animals were killed. As shown in table 1, the seminal vesicles of the animals receiving the adrenal compound weighed significantly more than those of the untreated animal and compared quite well with those of the animals receiving the male hormone. The prostate sizes (no weights were taken) fell into the same order as the seminal vesicle weights. Histologically, the seminal vesicles of all 5 animals were alike; i.e., there was, as would be expected in view of the dose given, no detectable stimulation of any of them over the castrate state. There was no apparent relative increase in muscle or connective tissue in the seminal vesicles of the treated animals. While the prostates of the rats receiving testosterone propionate showed histologic stimulation of the order of the dose, the prostates of the animals receiving the adrenal compound showed, paradoxically in view of their size stimulation, very slight if any histologic stimulation over the castrate state.

As an additional test an attempt was made to ascertain whether desoxycortico-

sterone acetate in 0.5 mg. and 1.0 mg. daily doses would blacken the bill of the English sparrow as does male sex hormone preparations (13). The smaller dose evoked no bill response, and the larger amount of oil (0.2 cc. daily) given with the larger dose proved to be lethal. Indeed the smaller quantity of oil (0.1 cc. daily) was by no means well tolerated, a factor which may have interfered with the eliciting of a response by the bill.

DISCUSSION

These observations show that desoxycorticosterone acetate is capable of exerting effects which are ordinarily attributed only to male sex hormone substances. While in general the response of the test characters was indistinguishable from the response of these structures to male hormones, the failure of the rat prostate to show histologic response is an exception for which we can at present offer no explanation. Histologic stimulation of the prostate is ordinarily produced by 0.3 B.U. of androgen and was, in fact, produced in these experiments by 5γ of testosterone propionate. Desoxycorticosterone thus may be said to resemble androsterone more than testosterone in action in that it is relatively more active on the comb than on the histology of the prostate.

Although the data presented are inadequate for any exact quantitative formulation, all the observations are in general accord with figures given: 1.0 mg. possesses approximately 0.3 B.U. of androgenic activity. Whether the androgenicity results from the conversion of desoxycorticosterone into some chemically related androgen or is inherent in the compound is, of course, an open question.

Any significance of the compound in the masculinizing action of the adrenal appears unlikely in view of its very weak androgenicity. However, it may be recalled that in mice with an experimental glandular imbalance Pfeiffer (14) has observed all the modifications which are produced by prolonged administration of large amounts of estrogen, despite the fact that the ovaries showed little evidence of excessive estrogen production.

Some interest attaches to the fact that desoxycorticosterone is even slightly androgenic in view of the fact that it is one of the very few compounds obtained from gland sources which have this action; others are testosterone, adrenosterone (9) and progesterone (15).

Desoxycorticosterone has rather an impressive list of capabilities. In addition to its activity as an essential adrenal cortical hormone, it will stimulate mammary development somewhat after the manner of the estrogens (16), it has progesterone-like activities (11, 17, 18), and is androgenic. The full physiological significance of a compound which has effects characteristic of so many hormones is a question of no little interest.

SUMMARY

The ability of desoxycorticosterone acetate to exert androgenic action has been tested in the capon, mouse and rat. Daily injections of 2 mg. into capons for 5 days resulted in an average increase of slightly more than 3 mm. in the length plus height of the combs. Daily injections of 1 mg. into castrated rats resulted in significant maintenance of the weight of the prostate and seminal vesicles but did not prevent castration changes in the histology of these organs. Administration of 0.5 mg. and 0.25 mg. to mice had results essentially like those of the larger amounts given to rats. The androgenic activity of the compound is thus approximately 1/30 as great as that of androsterone; and the compound resembles androsterone more than testosterone in being relatively more active upon the comb than upon the rodent accessories.

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EXPERIMENTS ON THE BIOLOGICAL PROPERTIES OF STILBESTROL AND STILBESTRYL DIPROPIONATE

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SINCE THE initial report of Dodds, Goldberg, Lawson and Robinson (1) on the preparation and estrogenic properties of stilbestrol, a number of workers have further compared the actions of this compound and its propionic and acetic acid esters with those of estrogens with a nucleus of the phenanthrene type. In the majority of experiments thus far published the two classes of compounds have been found to be qualitatively similar in their action.

These stilbene derivatives already have a rather extensive therapeutic use in Europe. They are being used in the United States in clinical investigations. However, before they are released for general clinical use more pharmacological and clinical studies are necessary in order to first establish their clinical safety and therapeutic value.

It was thought of interest, therefore, to repeat and extend certain of the earlier experiments on stilbestrol and to include work on the propionic acid ester. Such studies were deemed to be especially important in the case of these synthetic estrogens since it has previously been reported that some synthetic compounds may exhibit physiological actions which resemble those of naturally occurring substances but also evidence undesirable side effects. A consideration of the ester seemed indicated since most of the reports have dealt primarily with stilbestrol and because it is well known that relatively slight changes in the chemical structure of the various sex hormones cause them to have qualitative differences in their action.

The following reports are particularly relevant to the present investigation. Dodds, Lawson and Noble (2), and de Fremery and Geerling (3) found that stilbestrol induced growth of the immature rabbit uterus and sensitized it to progesterone. Noble (4) reported that the subcutaneous implantation of stilbestrol would inhibit body growth and development of the genitalia of male and female rats through suppression of the anterior pituitary. However, he found that the administration of chorionic gonadotropin stimulated the ovaries of stilbestrol-treated rats more than it did those of untreated controls.

The present communication represents a study concerned with the action of stilbestrol and stilbestryl dipropionate[1] upon (*a*) body growth, reproductive system, pituitary and adrenals of immature rats and (*b*) the immature rabbit uterus with especial regard to the ability of the stilbene derivatives to sensitize it to progesterone. Seventeen rabbits and 317 rats were used in these experiments. No attempt was made to study the tissues of the rats histologically since such large amounts of material were administered and since it was thought that there are as yet too few quantitative

[1] 4:4′-dihydroxy-alpha:beta-diethyl stilbene and 4:4′-dipropionyl-alpha:beta-diethyl stilbene.

data regarding the absorption and utilization of subcutaneous implants of crystalline material of this type. Experiments are now in progress for making a more quantitative comparison of the toxicity and physiological actions of these compounds with those of estrone. The results of this work will be submitted in another communication.

PROCEDURE AND RESULTS

Comparative Action of Stilbestrol, Stilbestryl Dipropionate and Estrone on the Immature Rabbit Uterus

Stilbestrol, stilbestryl dipropionate and estrone (international standard) were compared with regard to their ability to induce growth and development of the immature rabbit uterus and to sensitize it to progesterone by the McPhail technic (5). Different groups of immature female rabbits (3 per group) weighing between 1000 and 1600 gm. were given 15γ of each estrogenic substance in 3 divided doses on alternate days. Progesterone (0.75 I.U.) was administered in divided doses once per day on the succeeding 5 days and the animals were autopsied on the sixth day after the initiation of progesterone treatment. All injections were made intramuscularly in sesame oil. Uterine biopsies were made prior to treatment and after the estrogen had been given for purposes of comparison with autopsy material after the administration of progesterone.

In all animals the 3 estrogens produced a marked degree of growth in the myometrium and an endometrial development typical of that normally brought about by follicular activity in the mature animal. After the subsequent administration of progesterone, endometria of the secretory type resulted in all cases. The histological picture of the uterine biopsies made after the estrogens had been given was very similar regardless of the substance used. Likewise, the condition of the uteri of the different groups of rabbits at autopsy after progesterone treatment was much the same. Any variations were too slight to allow of our drawing conclusions as to differences in the qualitative or quantitative actions of the various estrogenic substances. The results indicated that under the conditions of these experiments both stilbestrol and stilbestryl dipropionate were as effective as estrone in promoting development of the immature rabbit uterus and in sensitizing it to progesterone.

Our results confirmed the reports of Dodds and his coworkers, and de Fremery, and Geerling regarding these actions of stilbestrol. Whereas Dodds et al. used relatively large amounts of stilbestrol and progesterone, we thought it advisable to compare the actions of the stilbene derivatives and estrone by following a standard method which involved the use of small quantities of material.

Since certain estrogens and androgens are known to exhibit a progestin-like acs tion, it was thought worth while to determine whether these stilbene derivative- would evidence such properties. Different groups of 3 immature female rabbits were therefore given dosages of 1.0 mg. of stilbestrol and 4.0 mg. of stilbestryl dipropionate in divided doses once per day for 10 days and were autopsied on the eleventh day of the experiment. All material was administered intramuscularly in sesame oil.

In all cases the uteri showed marked stimulation of the estrogenic type with no evidence of progestational proliferation. The results indicated that neither of these synthetic estrogens possessed progestin-like properties.

Effect of Stilbestrol and Stilbestryl Dipropionate upon the Body Growth, Reproductive System, Pituitary and Adrenals of Rats

Effect on body growth. Ten mg. of crystalline stilbestrol was given to 30-day-old animals of both sexes by subcutaneous implantation according to the method described by Parkes (6) and Deanesly and Parkes (7) for tablet implantation. In a like manner

implants of 10 mg. of stilbestryl dipropionate were made in a group of 30-day-old males. Groups of untreated animals of the appropriate age were used as controls. The method was similar to that used by Noble and seemed advisable since it would afford a basis for comparing our results on stilbestrol with his.

Body weights of all animals on long term experiments were taken daily. The females were autopsied on the 26th and the males on the 28th day after the initiation of the experiment. The data concerned with the effect of the synthetic estrogens upon body growth are included in table 1. It will be seen that stilbestrol produced a marked degree of growth suppression in both sexes. The inhibitory action was most pronounced in the week after implantation and progessively decreased during the remaining part of the experiment. These results are similar to those of Noble, but cannot be compared quantitatively since the age of the animals used in his experiments was not stated.

TABLE 1. THE EFFECT OF SUBCUTANEOUS IMPLANTATION OF 10 MG. OF STILBESTROL AND STILBESTRYL DIPROPIONATE UPON THE BODY GROWTH OF 30-DAY-OLD RATS[1]

No. of rats and sex	Drug	Ave. wt. at start of exp.	Ave. wt. gain per week				Ave. wt. at end of exper.	Ave. tot. weight gain
			I	II	III	IV		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
27 ♀	Stilbestrol	70	8	15	16	—	109	39
29 ♀	Controls	67	20	21	19	—	127	60
39 ♂	Stilbestrol	69	8	13	16	21	127	58
14 ♂	Stilbestryl dipropionate	68	4	9	10	9	100	32
21 ♂	Controls	65	16	29	23	28	162	96

[1] Weights of males were taken over a period of 4 weeks, those of females, 3 weeks.

As tested in the male, stilbestryl dipropionate proved to be a more effective growth inhibitor than stilbestrol and evidenced a more prolonged action. Like stilbestrol it suppressed the growth to a greater extent in the week after implantation. However, the weight gain in the following weeks was small and constant.

Cobet and his coworkers (8) reported the ester to be approximately one-third as potent an estrogenic agent as stilbestrol. As compared with stilbestrol on the basis of biological activity a smaller amount of the dipropionate was administered to the rats in our experiments. However, Dodds et al. (9) stated that the dipropionate has a more prolonged action than does stilbestrol. Therefore, it is probable that the more pronounced inhibition of growth effected by stilbestryl dipropionate was due to its greater duration of action.

Effect upon organ weights. The effect of stilbestrol and stilbestryl dipropionate upon the weight of the pituitary, reproductive system and adrenals is shown by the autopsy data which are listed in tables 2 and 3. Fresh tissue weights were used in all

TABLE 2. EFFECT OF SUBCUTANEOUS IMPLANTATION OF 10 MG. STILBESTROL UPON THE WEIGHT OF THE PITUITARY, ADRENALS AND GENITALIA OF FEMALE RATS. (STILBESTROL IMPLANTS MADE 26 DAYS PRIOR TO AUTOPSY)

Treatment	No. of rats	Age at autopsy	Ave. body weight	Average weight			
				Pituitary	Ovaries	Uteri	Adrenals
		days	gm.	mg.	mg.	mg.	mg.
Stilbestrol	20	56	120	8.5	13.1	150	35
Controls	20	56	137	7	22.2	113	34
Controls	20	30	64	3.1	13.35	24	19.5

TABLE 3. THE EFFECT OF SUBCUTANEOUS IMPLANTATION OF 10 MG. STILBESTROL AND STILBESTRYL DIPROPIONATE UPON THE WEIGHT OF THE PITUITARY, ADRENALS AND GENITALIA OF MALE RATS (IMPLANTS MADE 28 DAYS PRIOR TO AUTOPSY)

Treatment	No. of rats	Age at autopsy	Ave. body weight	Average weight				
				Pituit.	Seminal vesic.	Prostate	Testes	Adrenals
		days	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Stilbestrol	39	58	128	6.58	29	20	421	34
Stilbestryl dipropionate	14	58	100	8.1	47	10	232	31
Controls	21	58	162	5.8	90	102	2202	30
Controls	23	30	70	3.13	12	36	546	19

cases; the uteri being pressed between layers of paper toweling to remove excess fluid.

Treatment with the stilbene derivatives resulted in an increase in the pituitary weight. The differences between the adrenal weights of the groups of experimental animals and the controls of the same age were so slight that it was impossible to make any conclusions regarding the effect of the synthetic estrogens upon this organ.

A comparison of the autopsy data on the experimental and control rats shows that stilbestrol suppressed the development of the ovaries and that this compound as well as the dipropionate exerted an inhibitory action on the testes, prostate, and seminal vesicles of the male. One difference between the action of stilbestrol and stilbestryl dipropionate is shown by a comparison of the weights of the genitalia of the males treated with the two substances. The ester reduced the weights of the testes and prostate more than did stilbestrol, but caused less inhibition in the development of the seminal vesicles. The more pronounced inhibitory effect of the dipropionate upon the testes and prostate was probably due to its prolonged action on the anterior pituitary. The increased duration of action may also have allowed it to produce a direct stimulation of the seminal vesicles as has been reported for the natural occurring estrogens (10).

Qualitatively, the results of our experiments regarding the effect of stilbestrol upon body growth and organ weights are similar to those of Noble.

Effect upon the gonadotropic potency of the anterior pituitary. As a means of determining the gonadotropic potency of the pituitary, a total dose of 3 glands of the estrogen treated rats and 56 to 58-day-old controls was given to each animal used in the assay.[2] The pituitaries of the untreated 30-day-old animals of both sexes were assayed by administering doses of 1, 2 and 3 glands. It was thought that such a procedure would enable us to make a comparison of the gonadotropic potency of the pituitaries of the different groups of animals and might afford a basis for making a

[2] The method used in preparing the pituitaries for assay was similar to that described by Heller, Lauson and Sevringhaus (11) and Meyer, Mellish and Kupperman (12). At autopsy the pituitaries from the different groups of animals were combined separately and kept frozen until used. The glands were ground in a test tube by means of a glass rod. Water was added and the suspension further divided by flushing in and out of a syringe through a fine gauge needle. Phenol (0.5%) was added as a preservative. It was thought that this method was preferable to extraction or desiccation since it would avoid loss of material or alteration of the gonadotropic complex which might conceivably occur with chemical treatment. Furthermore, Heller et al. concluded from the results of their work that a more uniform response was obtained by using this procedure. In all pituitary assays the recipients were 22-day-old female rats.

All gonadotropic assays were made by administering the material subcutaneously in divided doses of 0.5 cc. twice per day for 3 days. The animals were autopsied on the morning of the 5th day, 96 to 100 hours after the first injection. The recipients used in any one assay were taken from at least 3 litters in order to minimize the effect which variability in response between litters might have upon the comparative value of the assay.

rough quantitative estimation of the degree to which the hypophysis had been inhibited by the estrogens.

TABLE 4. THE EFFECT OF STILBESTROL AND STILBESTRYL DIPROPIONATE UPON THE GONADOTROPIC POTENCY OF THE RAT PITUITARY

Donors		Recipients		
Drug implanted at 30 days of age (10 mg.)	Age (days) and sex	No. of pituitaries injected per rat	Ovaries, average weight	No. of immature female rats
			mg.	
Stilbestrol	58♂	3	55	11
Stilbestryl dipropionate	58♂	3	9	4
Controls	58♂	3	85.33	6
Controls	30♂	1	27.16	6
Controls	30♂	2	40.83	6
Controls	30♂	3	51.50	6
Stilbestrol	56♀	3	12.80	6
Controls	56♀	3	29.20	6
Controls	30♀	1	16	5
Controls	30♀	2	30	5
Controls	30♀	3	63	5
		0	13.35[1]	20

[1] Untreated controls 26 days of age.

The data in table 4 show that stilbestryl dipropionate suppressed the gonadotropic potency of the pituitary of the male to such an extent that no activity was evidenced from the administration of 3 glands. Stilbestrol exhibited a similar degree of pituitary inhibition in the female. This reduction in the potency of the pituitaries of these 2 groups of animals is correlated with the suppression in gonad development after the estrogen treatment (table 2, 3). However, 3 pituitaries from stilbestrol-treated males produced an average ovarian weight of 55 mg. as compared with 85.33 mg. and 51.50 mg. which resulted from the administration of 3 glands from 58 and 30-day-old animals respectively.

In view of the fact that the weights of the genitalia of the stilbestrol-treated males were markedly less than those of males of the same age, it was rather surprising to find the pituitary so potent. A comparison of the autopsy data and pituitary assays shows that the weights of the genitalia and gonadotropic potency of the pituitary of the stilbestrol-treated animals were similar to those of 30-day-old animals. These data suggest that stilbestrol prevented the pituitary from increasing in gonadotropic potency and effected a reduction in its gonad-stimulating properties to a level similar to that found at 30 days of age. However, other mechanisms such as changes in the release and elimination of the gonadotropic complex and/or gonadotropic antagonist may have been involved in producing these results.

Effect of Gonadotropin from Human Pregnancy Urine upon the Ovarian Weight of Stilbestrol-treated Rats

As an indirect means of measuring the gonadotropic activity of the anterior pituitary of stilbestrol-treated animals and in order to determine whether the ovaries were capable of responding to gonadotropic material, 0.5 mg. of an extract of human pregnancy urine (PU) containing the anterior pituitary-like gonadotropic principle was administered to one group of animals which had previously been treated with stilbestrol. For purposes of comparison groups of untreated animals were given a similar amount of the pregnancy urine extract.

TABLE 5. THE EFFECT OF PU EXTRACT UPON OVARIAN WEIGHT OF STILBESTROL-TREATED RATS. (THE STILBESTROL IMPLANTS WERE MADE AT 30 DAYS OF AGE AND THE PROLAN TREATMENT WAS BEGUN 5 DAYS PRIOR TO AUTOPSY)

Treatment	No. of rats	Age at autopsy	Ave. weight of ovaries
		days	*mg.*
Stilbestrol 10 mg.	20	56	13.1
Stilbestrol 10 mg. plus PU ext. 0.5 mg.	7	56	27.14
PU extract 0.5 mg.	9	56	41.64
Untreated controls	20	56	24.2
PU extract 0.5 mg.	14	34	39.57
Untreated controls	20	30	13.35

That the ovaries of the stilbestrol-treated rats were capable of responding to a gonadotropin is shown (table 5) by the fact that the PU increased the weight of the ovaries to 27 mg. as compared with a weight of 13 mg. in the animals which had received stilbestrol only. Whereas the weight of the ovaries in the stilbestrol-PU-treated group was not as large as that of the 56-day-old PU-treated group, yet the amount of increase in the ovarian weights over that of the appropriate controls for each group was similar (14 and 17 mg. respectively). In neither of these groups did the PU increase the weight of the ovaries as much as it did in the 30-day-old animals.

If, as is commonly thought, the APL factor acts primarily through interaction with the gonadotropic complex of the anterior pituitary and by augmentation of the pituitary gonadotropins, these data would indicate that the gonadotropic potency of the hypophysis of the untreated 30-day-old female animal is greater than that of one 51 days old. Such a conclusion is supported by a comparison of the pituitary assays of untreated animals of the 2 age groups (table 4). Two glands from the 30-day-old rats were equivalent in gonadotropic potency to 3 glands from the older age group. If the assumed method of action of the gonadotropin is correct, a greater response to the PU might have been expected in the younger rats. Such a response was realized.

The same line of reasoning would lead one to think that the gonadotropic activity of the pituitaries of the stilbestrol-treated animals and the untreated controls of the same age was the same. However, the assay data on the pituitaries of these 2 groups do not support such a conclusion since 3 glands from the estrogen-treated group evidenced no stimulus to the ovaries of the recipients whereas 3 glands from the untreated 56-day-old animals produced a marked increase in ovarian weight. The data are insufficient to allow of our making any definite conclusion as to the mechanism responsible for such results.

Our results confirm those of Noble in that the ovaries of stilbestrol-treated animals are capable of being stimulated by PU. In our experiments, however, the response was not of the same order as that obtained by Noble since his data show an increase in weight much greater than that of controls of the same age.

SUMMARY

When tested by the McPhail technic, stilbestrol and stilbestryl dipropionate were

at least as effective as an equal weight of estrone in producing growth and development of the immature rabbit uterus and in sensitizing it to progesterone.

Neither stilbene derivative exhibited progestin-like action when administered to immature female rabbits in doses of 1.0 and 4.0 mg. respectively over a period of 10 days.

Subcutaneous implants of 10.0 mg. of stilbestrol and stilbestryl dipropionate were made in 30-day-old rats. The animals were autopsied at 56 to 58 days of age. Stilbestrol inhibited body growth in animals of both sexes. In males the dipropionate evidenced a more effective inhibition of body growth and a more prolonged action than did stilbestrol. Stilbestrol inhibited the growth of the gonads of both sexes as well as the seminal vesicles and prostate. Stilbestryl dipropionate caused a greater suppression of the testes and prostate than did stilbestrol but inhibited the seminal vesicles to a less degree.

Stilbestrol markedly reduced the gonadotropic potency of the anterior pituitary of the female but showed relatively little inhibitory action on the male hypophysis. Stilbestryl dipropionate, however, caused a definite reduction in the gonad-stimulating power of the male pituitary.

Chorionic gonadotropin increased the ovarian weight of rats previously treated with stilbestrol. The weight increment was equal to that produced by PU in untreated animals of the same age but less than that resulting from its administration to immature animals.

Treatment with the stilbene derivatives increased the weight of the pituitary, but did not cause a significant change in the weight of the adrenals.

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THE INFLUENCE OF ESTRIOL, ESTRADIOL AND PROGESTERONE ON THE SECRETION OF GONADOTROPIC HORMONES IN PARABIOTIC RATS[1]

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IN PREVIOUS quantitative studies from this laboratory it has been shown that the ovarian response of the non-castrated partner of parabiotic rats is a sensitive and reliable indicator of the quantity of gonadotropic hormone released into the circulation of the rats by the pituitary gland of the castrated partner. It was demonstrated that crystalline estrone (1), estrin from the amniotic fluid of cattle (2) and testosterone, testosterone propionate and dehydroandrosterone (3) were effective in preventing the hypersecretion of gonadotropic hormone that occurs following castration of either male or female rats. When progressively greater quantities of the gonadal hormones were injected into the castrated partner the size of the ovaries of the normal partner was progressively decreased. If sufficient hormone was injected the ovaries were no larger than those of non-castrated controls. It was concluded from these studies that the gonadal hormones prevented the hypersecretion of the gonadotropic hormones, the degree of suppression being directly proportional to the amount of hormone injected. Studies made upon single animals likewise indicate that gonadal hormones prevent castration hypersecretion of the gonadotropic complex (4, 5, 6).

The present communication is an extension of the previous studies to include the effect of estriol, estradiol and progesterone in preventing castration hypersecretion in parabiotic rats.

MATERIALS AND METHODS

This study includes observations upon a total of 186 pairs of rats, including 39 untreated control pairs run simultaneously with the treated pairs, and 147 experimental pairs treated with varying doses of estriol, estradiol and progesterone. In addition we wish to present a summary of all our data on untreated control pairs obtained since we began working with parabiotic rats, bringing them up to date as a 'combined control' group.

The operative procedure used in uniting the animals was that described by Bunster and Meyer (7), except that metal skin clips were used in closing the skin wounds instead of silk sutures. All operations were performed under sterile conditions and under ether anesthesia as in our previous studies.

Litter mates weighing 70 gm. or more were used in all cases. The animals were united at 31 to 33 days of age and the right partner was gonadectomized at the same

[1] Supported in part by a grant-in-aid from the Wisconsin Alumni Research Foundation and assistance given by the University of Wisconsin WPA Natural Science Project.

time. The right partner was injected subcutaneously, within 2 to 3 hours following the operation, with 0.01 cc. of corn oil in which the estrogen or progesterone was dissolved. The injections were made once daily for 10 days with autopsy on the morning of the 11th day of parabiosis. The ovaries were dissected free of extraneous tissue and the gross qualitative response and weight to the nearest milligram recorded.

In determining the dosage of hormone which prevented castration hypersecretion, a dosage was selected which was calculated to have about the same estrogenic activity as the inhibiting dosage of estrone previously determined in parabiotic rats (1). If no ovarian stimulation was obtained in the pairs receiving the first dosage, this amount of hormone was halved and injected into a second series of animals. This method of halving the previous amount of hormone was continued until a level was found at which some or all of the pairs showed ovarian stimulation.

Table 1 shows the results obtained with the different amounts and kind of hormones together with the mean ovarian weights and their standard error and the ovarian weight distribution. By careful sterile precautions we have been able to practically eliminate infection, therefore the data are unselected and include all the pairs that were placed on the experiment.

RESULTS AND DISCUSSION

It will be seen from the results given in table 1 that as the dosage of hormone was increased in the experimental pairs, the ovarian weights decreased until a level was reached which corresponded with the ovarian weights of normal rats of the same age. When this obtained in all the pairs of the group, we considered that dosage the one which prevented completely castration hypersecretion of gonadotropic hormone. On this basis, the amount of estriol necessary for complete suppression in female-female pairs was 1.5γ per day, while in female-male pairs 10γ per day, or 6.6 times as much, was necessary. Using estradiol, 0.025γ per day was required for suppression in female-female pairs, while 0.15γ per day was needed in female-male pairs. With this estrogen, 6 times as much was needed in female-male pairs as in female-female pairs.

When progesterone was injected into female-female pairs only partial suppression was obtained with 500γ per day, while 1000γ per day gave essentially complete suppression. Seven pairs which received 1 rabbit unit of progestin per day, prepared by extraction of sow ovaries, showed practically complete suppression. It is not absolutely certain, however, that all traces of estrogen were removed during the process of extraction; therefore we consider only the pairs which received crystalline progesterone in our comparisons. There was only one pair in the group which received 1000γ per day that showed any ovarian stimulation. This fact indicates that this dosage of progesterone probably is not sufficient to suppress the castration hypersecretion of all female rats. Selye, et al. (8) and Phillips (9) have shown that progesterone will prevent the occurrence of estrous cycles in the female rat. The former injected 4 mg. over a period of 12 days, and obtained cessation of the cycle, while the latter found that 1.5 mg. of crystalline progesterone per day was the minimum dosage necessary to inhibit the estrous cycle.

The large quantity of progesterone required to prevent castration hypersecretion shows that this hormone is very inefficient in suppressing pituitary secretion, it being approximately 40,000 times less effective than estradiol, 5,000 times less effective than estrone and 666 times less effective than estriol. Since estradiol, estrone and estriol are so much more effective than progesterone in suppressing the secretion of gonadotropic hormone, it does not seem necessary to postulate that progesterone per se is of much importance in this regard in the normal cycle. It would also seem that estradiol, because of its strength in suppressing gonadotropic hormone secretion,

may be the most important female gonadal hormone in the control of the cyclic function of the ovary.

In a previous study it was shown that 0.2γ of estrone per day was necessary for complete suppression of castration hypersecretion in female-female pairs (1). The present results show that in female-female pairs 1.5γ of estriol and 0.025γ of estradiol per day are necessary for complete suppression. Or to express it differently, estrone is 7.5 times as effective as estriol and estradiol is 8 times as effective as estrone. In female-male pairs, 1γ of estrone per day has been found to be necessary for complete suppression, while in this study 10γ of estriol and 0.15γ of estradiol per day are required, or estrone is 10 times as effective as estriol and estradiol is 6.6 times as effective as estrone. Therefore, the difference in activity between estrone and estriol when determined in female-female pairs is approximately the same as when determined in female-male pairs, i.e., 7.5 and 10 times respectively. Likewise, there is good agreement between the difference in the activity of estrone and estradiol when ascertained in female-female pairs and when determined in female-male pairs, i.e., 8 and 6.6 times respectively. It has been reported that estradiol is from 3 to 6 times as potent as estrone (10), and that estrone is from 90 to 100 times as potent as estriol (11, 12) in their ability to produce vaginal cornification in adult spayed female rats. A comparison of the ability of estriol, estrone and estradiol to prevent castration hypersecretion of the pituitary gland of the rat demonstrates that the order of their effectiveness in this regard is the same as that found for their effect on the rat vagina. We do not find, however, that the difference between the activity of estriol and estrone in preventing castration hypersecretion is as great as the difference in activity as determined by their effect upon the vagina.

The combined control data from the parabiotic rats (table 1) shows that there is a difference of only 11 mg. between the mean ovarian weights of the female-female and the female-male pairs. The number of animals in each group is large enough that the ovarian weights obtained would seem to be a true representation of the amount of gonadotropic hormone released by the castrate female and the castrate male pituitary gland. These data suggest that the amount of gonadotropic hormone secreted by the pituitary gland of the male rat during the first 10 days of castration is very little if any greater than that secreted by the castrate female rat pituitary. The data reported by Evans (13) shows that the pituitary gland of the castrate male rat does not contain much more gonadotropic activity than the pituitary gland of the castrate female. However, in the present study and in the one in which androgens were used (3) it is evident that the male requires much more of a given estrogen or androgen to prevent hypersecretion of gonadotropic hormone after castration than does the female. We believe that this does not necessarily mean that the male gland secretes more gonadotropic hormone than the female gland but that there is a greater predisposition on the part of the secretory cells of the male pituitary gland to secrete gonadotropic hormone than there is in the case of the cells of the female gland, and that it requires more androgen or estrogen to totally inhibit this predisposition.

We have previously reported that approximately 13% of the control pairs failed to show any ovarian stimulation. Of the present combined control group, 11% of the pairs did not show any ovarian enlargement. Such pairs are grossly indistinguishable from other control or injected pairs in the rate of healing and absence of infection. This failure of response may indicate that the pituitary gland of every rat does not secrete an increased amount of gonadotropic hormone following castration.

In our previous reports (1, 3) concerned with the effect of estrogens and androgens on the prevention of castration hypersecretion of gonadotropic hormone we

have not discussed the type of gonadotropic hormone which we believe to be secreted by the pituitary gland of the castrated male or female rat. We purposely have avoided such a discussion since we did not have sufficient data which had a direct bearing on the question. However, we now have evidence from studies of hypophysectomized parabiotic rats united in parabiosis with castrated rats and from rats treated with estrogens before being united in parabiosis. The data resulting from these studies will be published subsequently and they, together with data presented in this report, provide the basis for the following statements. (*a*) During the first 10 days of castration the pituitary gland of the male and female rat secretes only follicle stimulating hormone. (*b*) The ovaries of the intact rat in parabiosis with a castrated male or female contain corpora lutea because the estrogens secreted by the

TABLE I

Hormone	Sex of pairs	Dose per day (γ)	No. pairs	Mean ovarian weight ±S.E.	Ovarian weight distribution (mg. 0–20	21–34	35–49	50–64	65–79	80–94	95–109	110–124	125–214
Estriol	♀♀	0.1	6	38± 10.5	1	3			2				
		0.3	7	22± 5.3	6			1					
		0.5	6	14± 1.7	6								
		1.0	5	17± 2.0	3	2							
		1.5	4	13± 1.8	4								
		3.0	6	13± 0.8	6								
		4.5	5	16± 0.8	5								
	♀♂	4.5	5	86± 14.0			1			2	1	1	
		6.0	7	41± 11.9	3	1		1	1		1		
		8.0	7	19± 5.4	6			1					
		10.0	5	15± 0.4	5								
		15.0	4	12± 0.8	4								
Estradiol	♀♀	0.01	7	46± 14.9	3	1			1	1		1	
		0.0175	7	13± 1.8	6	1							
		0.025	6	15± 2.0	6								
		0.05	5	13± 0.4	5								
	♀♂	0.05	6	52± 15.7	2		1		2		1		
		0.075	6	23± 5.2	3	2	1						
		0.10	5	22± 2.3	1	4							
		0.15	5	14± 0.9	5								
Progesterone	♀♀	100	18	42± 8.0	8	2	1	3		3		1	
		1000	8	20± 7.1	7				1				
		1 RB.U.	7	17± 2.7	6	1							
Controls Group 5	♀♀	—	24	85± 10.9		7	2	1	4		1	3	6
	♀♂		15	88± 13.5	1	2	3			1	3	1	4
Combined Controls	♀♀	—	59	80± 5.8	4	9	6	3	11	3	6	6	11
	♀♂		59	91± 7.1	9	3	6	2	2	9	6	6	16

ovaries release luteinizing hormone from the pituitary gland of the intact rat. (*c*) The quantities of estrogens and androgens necessary to prevent castration hypersecretion of gonadotropic hormone in male and female rats either prevents the production or release of follicle stimulating hormone and at the same time increases the production of, or the release of, luteinizing hormone. The luteinizing hormone from such a castrated parabiont is not able to bring about the formation of corpora lutea in the intact parabiont because of the undeveloped condition of its ovaries.

These conclusions are in agreement with those developed by Hellbaum (personal communication) who has been investigating the type of gonadotropic hormone present in the pituitary glands of castrated male and female rats and the effect of androgens and estrogens on the type secreted. Our conclusions, which have been developed as a result of studies on castrate rats, are also essentially in agreement with those formed by Fevold et al. (14, 15), Hohlweg (16) and Lane (17) who studied the effect of the interaction of the two gonadotropic hormones and the influence of estrogens on the secretion and interaction in individual normal rats.

SUMMARY

Observations on a total of 186 pairs of parabiotic rats are presented. Of these 39 are untreated controls and 147 are experimental pairs that received varying amounts of estriol, estradiol and progesterone. In addition, a combined control group of 118 pairs is presented which summarizes all of our data on untreated control pairs.

It has been determined in 31- to 41-day-old female-female pairs that 0.025γ of estradiol, 1.5γ of estriol and 1000γ of progesterone per day are required to prevent the hypersecretion of gonadotropic hormone that occurs following castration of the female rat. In female-male pairs it was determined that 0.15γ of estradiol and 10γ of estriol per day are necessary to prevent the hypersecretion of gonadotropic hormone in the male rat. These results are compared with those previously reported when estrone was used. It is shown further that the order of effectiveness of the estrogens used in preventing castration hypersecretion, i.e., estradiol > estrone > estriol, is the same as the order of their effectiveness in producing vaginal cornification.

Because of the slight difference in the ovarian weights of 59 female-female and 59 female-male control pairs, it is concluded that the male pituitary gland secretes very little, if any more, gonadotropic hormone during the first 10 days of castration than does the female pituitary. A discussion of the type and interaction of the pituitary gonadotropic hormones in parabiotic rats is also included.

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SYMPOSIUM ON CARBOHYDRATE METABOLISM*

GLYCOGEN BREAKDOWN AND SYNTHESIS IN ANIMAL TISSUES

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THE ENZYMES concerned with the breakdown and synthesis of glycogen in liver and muscle play a fundamental rôle in carbohydrate metabolism. The enzymatic breakdown leads to the formation of blood sugar in liver and of lactic acid in muscle. Various substances (insulin, epinephrin, ergotoxine, adreno-cortical and anterior pituitary hormones and others) influence enzyme activity in the cell. When one investigates changes in liver and muscle glycogen under various experimental conditions, and there exists a very large literature on this subject, one is really measuring variations in the rate of activity of cellular enzymes. Yet, until recently, information regarding the enzymes which act on glycogen was very scanty. It had not been recognized that in spite of the different end products of glycogen breakdown in liver and muscle the initial stages of glycogen degradation are the same in these two tissues and in other tissues as well. It was also unknown that the first step in the breakdown of glycogen involves a reversible enzymatic process.

Progress in this field came from a study of individual enzymatic reactions outside the organization of the cell. Experiments carried out on intact animals or organs give an all-over result of a series of enzymatic reactions, but they do not reveal the intermediate steps involved. Even in tissue extracts the integration of the enzymatic processes can be so complete that the intermediate steps are not recognizable. In order to bring them to light, it is necessary to separate from each other the enzymes present in tissue extracts or to use specific poisons which block enzymatic processes at a desired point.

If a rather detailed analysis of certain enzymatic processes is given in this paper, it is with the hope that this approach will eventually be of value for an understanding of the mechanism of action of insulin, epinephrin and other hormones.

Diastase and Phosphorylase

One of the chief reactions concerned with the regulation of blood sugar level may be represented thus: glycogen $\rightleftarrows$glucose. Up to now it has been generally assumed that in the liver a diastase breaks down glycogen and that its synthesis from glucose involves 'polymerization,' a process catalyzed

* The following four papers were read in a symposium sponsored by the American Physiological Society, at Toronto, Canada, April 29, 1939. Chairman: Professor C. H. Best.

either by diastase or some unknown enzyme. The fact that dextrins of varying chain lengths and maltose are the main products of diastatic activity in vitro and that glucose appears in large amounts only after long periods of incubation (1, 2) was difficult to reconcile with the fact that liver and blood contain none of these intermediates. It made necessary the assumption that the kinetics of the diastase in the cell are of an entirely different nature from those in the test tube.

There have been reports which emphasized the low diastase content of extracts prepared from perfused livers (3), but since other enzymes capable of breaking down glycogen had not been shown to occur in the liver, little attention was paid to this finding. In recent experiments (4) it was shown that the diastase content of liver extracts of various species is correlated with the diastase content of the blood, and this varies greatly in different species. In rabbits the blood diastase is relatively low and extracts prepared from organs (liver, heart, muscle, brain) previously perfused with Ringer's solution are almost devoid of diastatic activity. These extracts were subjected to dialysis in order to remove inorganic and organic phosphates and coenzymes. When glycogen, inorganic phosphate and a trace of muscle adenylic acid were added, a rapid breakdown of glycogen occurred, while the omission of either inorganic phosphate or of adenylic acid prevented the breakdown of glycogen (5). The enzyme found in these extracts has been named (glycogen)-phosphorylase. It has also been found in yeast (5) and further work will probably show that it is widely distributed in animal and plant tissues.

Initial Stages of Glycogen Breakdown

The phosphorylase initiates the degradation of glycogen. It also acts on starch and some higher dextrins, but is without effect on maltose, glucose and other sugars. It acts under anaerobic conditions and is not inhibited by iodoacetate and fluoride in moderate concentrations. Phlorhizin and phloretin inhibit the enzyme (6). Adenylic acid (adenine-ribose-5-phosphoric acid) functions as coenzyme; if it is removed by electrodialysis of the extracts, breakdown of glycogen does not occur. Addition of 0.0001 M adenylic acid restores the activity of such extracts (7).

The phosphorylase catalyzes the following reaction in a reversible manner.

$$\text{glycogen} + H_3PO_4 \rightleftarrows \text{glucose-1-phosphate} \quad (1)$$

The reaction to the right causes a disappearance of inorganic phosphate, while the reaction to the left is associated with the liberation of inorganic phosphate. Whether the reaction goes in one direction or in the other depends on the relative concentrations of inorganic phosphate and of glucose-1-phosphate. At equilibrium the concentration of esterified phosphate is about one-fifth of the concentration of inorganic phosphate. It follows that the reaction to the right will soon come to an end if there is no enzyme present which removes glucose-1-phosphate. Such an enzyme has been found in all tissue extracts and is referred to as phosphoglucomutase (8); it catalyzes the following reaction

$$\text{glucose-1-phosphate} \rightarrow \text{glucose-6-phosphate} \quad (2)$$

In this reaction the phosphate group migrates from carbon atom number 1

to 6. Experiments with radioactive phosphorus (9) have given definite proof that this is an intramolecular migration. Mg^{++} ions (in concentrations in which they occur in muscle, namely 0.005 M) and Mn^{++} and Co^{++} ions in 0.0005 M concentration accelerate the activity of this enzyme up to 20 times. Glucose-1-phosphate does not accumulate in tissue extracts if Mg^{++} ions are present. If these ions are removed by electrodialysis of the extracts, glucose-1-phosphate accumulates but with time this ester disappears again, because reaction 2 can also occur, though at a much slower rate, in extensively dialyzed extracts (8). Reaction 2 could not be reversed by the action of the phosphoglucomutase. The only other step in the formation of lactic acid which so far has not been reversed anaerobically is the dephosphorylation of phosphopyruvic acid. There are some indications that both reactions are reversible under aerobic conditions.

Tissue extracts contain still another enzyme, Lohmann's (10) isomerase, which catalyzes the following reaction

glucose-6-phosphate $\rightleftarrows$ fructose-6-phosphate ..... (3)

At equilibrium there is about 80% of the glucose and 20% of the fructose ester present. The hexosemonophosphate isolated by Robison from yeast and by Embden from muscle contains these two esters in the above proportions and is referred to as equilibrium ester. This ester is a normal constituent of resting muscle and is the end product of glycogen breakdown in dialyzed extracts of muscle, heart and brain. If one measures simultaneously in such extracts the disappearance of glycogen and the accumulation of equilibrium ester, one finds a very close agreement at all periods of observation. In liver extracts, on the other hand, much more glycogen disappears than hexosemonophosphate accumulates. This difference makes it necessary to consider liver and muscle separately.

Glucose Formation from Glycogen in Liver

When the presence of phosphorylase in liver was first observed (5), its participation in blood sugar formation immediately suggested itself. This suggestion gained experimental support when it was found (11) that extracts from perfused rabbit livers which showed marked phosphorylase activity were almost free of diastatic activity. (The latter was measured by determining the disappearance of glycogen in dialyzed extracts without addition either of inorganic phosphate or of adenylic acid but in the presence of Cl ions which activate diastase.) Furthermore, when fermentable sugar (glucose) was determined in addition to hexosemonophosphate, the sum of the two substances accounted completely for the glycogen which disappeared.

Glucose-1- and glucose-6-phosphate, when added to liver extract, are split to glucose and inorganic phosphate. Liver, in contrast to muscle, contains a phosphatase which acts on the hexosemonophosphate formed from glycogen by the phosphorylase. The liver phosphorylase could be separated from the phosphatase by adsorbing it on aluminum hydroxide. In the enzyme solution obtained by elution of the phosphorylase with secondary phosphate the disappearance of glycogen was balanced by hexosemonophosphate formation and no fermentable sugar was formed.

The combined action of the phosphorylase and phosphatase explains the mechanism of blood sugar formation from glycogen in the liver. Although these enzymes are incompletely extracted and are unstable in liver extracts, rates of formation of fermentable sugar amounting to 2 gm. per 100 gm. of liver per hour have been observed, which are adequate for blood sugar formation under physiological conditions (4).

Some evidence has been obtained that this enzyme system is active in intact liver cells. Addition of phlorhizin in 0.01 M concentration almost completely suppresses the activity of the phosphorylase in liver and muscle extracts, so that practically no glycogen disappears, while it has only a slight effect on diastase. When liver slices prepared from perfused livers of rabbits and rats were shaken in oxygenated Ringer's solution, glycogen disappeared and fermentable sugar was formed. The glycogen breakdown was markedly inhibited by phlorhizin in 0.01 M concentration. Ostern and Holmes (12) reported recently an inhibition of glycogen breakdown in hashed liver amounting to 70% in the presence of phlorhizin, and they offered other experimental evidence which supports the idea that the chief, if not the only, route of glycogen breakdown in the liver is by way of glucose-1-phosphate.

Glucose-1-phosphate and Equilibrium Ester in Muscle

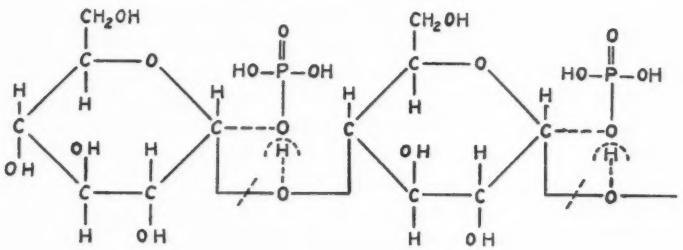
The study of hexosemonophosphate formation was originally begun on intact muscle. Eggleton and Eggleton (13) Davenport and Sacks (14) and Lohmann (15) showed that during stimulation of muscle a phosphate ester which is difficult to hydrolyze in acid accumulates; it was assumed to be the equilibrium ester. Cori and Cori (16) devised a quantitative method in which this ester is separated from other phosphate esters and reducing substances and is determined by its reducing power for alkaline copper or ferricyanide solution and its organic P content. With this method the formation of hexosemonophosphate was investigated in mammalian and frog muscle under a variety of experimental conditions which included the administration of epinephrin and insulin (17) and various forms of stimulation (18). In other experiments an attempt was made to determine the fate of hexosemonophosphate when it disappeared under anaerobic and aerobic conditions (19). Finally the hexosemonophosphate changes were correlated with simultaneously occurring changes in other phosphate fractions and in lactic acid content of muscle (20).

Following a short tetanic stimulation up to 50% of the glycogen which disappeared was present as hexosemonophosphate while the remainder was present as lactic acid (18). Traces of glucose (21) and pyruvic acid (22) were also formed. Since phosphocreatine decreased it was necessary to find out whether this was connected with the hexosemonophosphate formation. When epinephrin was injected into rats, the hexosemonophosphate content of muscle rose, but there occurred no change in phosphocreatine and the inorganic phosphate decreased (23). These same changes could be demonstrated more clearly when thin frog muscles were immersed in Ringer's solution containing epinephrin in a concentration of 1 to 10 million. The decrease in inorganic phosphate and increase in hexosemonophosphate occurred in equivalent proportions and there was no significant change in ohter phosphate fractions and only a slight increase in lactic acid (24). From these

and other findings it was concluded that the mechanism of hexosemonophosphate formation in muscle consists in an esterification of glycogen with inorganic phosphate, but no inkling was obtained that the equilibrium ester is not the primary product of this reaction.

A participation of phosphocreatine in hexosemonophosphate formation from glycogen was definitely excluded when it was found that the ester is formed in muscle (and other tissue) extracts which contain no phosphocreatine. It has been shown (cf. 25) that the breakdown of phosphocreatine during contraction is connected with the rephosphorylation of adenylic acid which is formed when adenosinetriphosphate transfers its mobile phosphate groups to fructose-6-phosphate to form fructose-1, 6-diphosphate (Harden-Young ester).

Since it seemed doubtful that an insight into the mechanism of hexosemonophosphate formation from glycogen could be gained by continuing experiments with intact muscle, it was decided to work with a simpler system. Frog muscle was finely minced with scissors and extracted several times with large volumes of cold distilled water. This treatment removed inorganic and organic phosphates and coenzymes, but left considerable amounts of glycogen and enzymes in the muscle residue. When the muscle



residue was suspended in phosphate buffer only small amounts of inorganic phosphate disappeared. Addition of minute amounts of adenylic acid greatly enhanced the disappearance of inorganic phosphate. The product formed after 3 hours of incubation was the equilibrium ester. When a time curve was made and the method of Cori and Cori for the determination of hexosemonophosphate was applied, it was noted that after short periods of incubation much more organic phosphate was present than corresponded to the reducing power. The formation of an intermediary product between glycogen and equilibrium ester was suspected and its isolation undertaken (26). The chief difficulty was its separation from the equilibrium ester which was finally accomplished by fractional crystallization of the brucine salts. After conversion to the barium salt the elementary analyses for C, H, P and Ba agreed with that of a hexosemonophosphate. It differed from other hexosemonophosphates by its lack of reducing power, the extreme ease with which it was hydrolyzed in weak acids (yielding equivalent amounts of glucose and inorganic phosphate) and the high optical rotation ([α]D+120° for the free acid). The compound was assumed to be α-glucose-1-phosphate and this assumption was verified by synthesis (27). Its formation from glycogen is illustrated in the following diagram.

The glycogen molecule, which consists of an α-glucosidic chain, is disrupted by esterification of each glucose unit with phosphoric acid on carbon atom number one, a process which has been named 'phosphorolysis' (25). In this enzymatic process the phosphoric acid molecule may be pictured as playing the same rôle as the water molecule in the 'hydrolysis' of glycogen by diastase.

Further experiments were undertaken with muscle extract. It was found that unless the extract was extensively dialyzed, glucose-1-phosphate did not accumulate. In this way the accelerating effect of Mg^{++} ions on reaction 2, the conversion of glucose-1- to glucose-6-phosphate, was detected. A method was described which permits the preparation of about 0.5 gm. of the barium salt of glucose-1-phosphate with 100 cc. of dialyzed extract. Kiessling (28) described an enzymatic method in which the glucose-1-phosphate is obtained as the crystalline dipotassium salt. A synthetic method which permits the preparation of the compound on a larger scale was described by Cori, Colowick and Cori (27). The synthetic product was shown to be identical with the natural product in all chemical and biological properties. Mannose-1- and galactose-1-phosphate have also been prepared synthetically (29).

In their experiments with muscle extract Parnas and Baranowski (30) and Ostern and collaborators (31) observed the formation of equilibrium ester from glycogen and inorganic phosphate and the formation of hexosediphosphate from equilibrium ester and adenosinetriphosphate. Since their extracts remained active when dialyzed, they concluded that a coenzyme was not necessary for the formation of equilibrium ester. Later Parnas and Mochnacka (32) obtained inactive extracts by dialyzing them more thoroughly and they were able to reactivate them by addition of adenylic and of inosinic acid. Cori, Colowick and Cori (7), Kendal and Stickland (33) and Bauer and collaborators (34) obtained only slight or no effects with inosinic acid. The difficulty of removing the last trace of adenylic acid by dialysis will be appreciated when it is realized that addition of 0.00003 M adenylic acid to an inactive extract produces a marked effect (7). When increasing amounts of adenylic acid are added to such an extract (which must be free of adenylic deaminase) (35) a typical coenzyme-activity curve is obtained. Adenosinediphosphate is about one-half and inosinic acid one-thirtieth as active as adenylic acid. Adenosinetriphosphate (in extracts free of pyrophosphatase, an enzyme which converts adenosinetriphosphate to adenylic acid), cozymase, yeast adenylic acid (adenine-ribose-3-phosphoric acid), and adenosine are inactive.

It was to be expected that if adenylic acid is the coenzyme of the phosphorylase it would be necessary for reaction 1 in both directions, that is, not only for the formation of glucose-1-phosphate from glycogen but also for the synthesis of glycogen from glucose-1-phosphate. This was found to be the case with purified phosphorylase prepared from extracts of muscle (36), heart, brain and liver (4). Glycogen synthesis did not take place, unless adenylic acid was added. Inosinic and adenosinetriphosphoric acid could not be substituted for adenylic acid.

The manner in which adenylic acid participates in reaction 1 is still under investigation. Other phosphorylations, e.g. that of creatine, involve the following reactions.

adenylic acid + $2H_3PO_4$ = adenosinetriphosphate

adenosinetriphosphate + 2 creatine = 2 phosphocreatine + adenylic acid

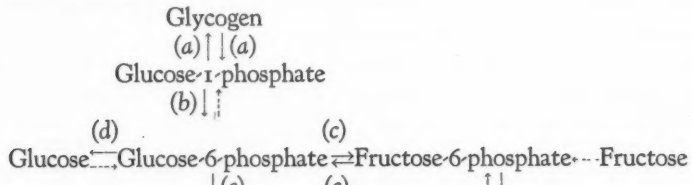
It is improbable that an analogous reaction takes place in the phosphate transfer to glycogen, because adenosinetriphosphate and adenosinediphosphate (7) when added in large amounts, do not donate their mobile phosphate groups to glycogen.

Since an oxidation of glucose-1-phosphate has not yet been demonstrated, the equilibrium ester is the first degradation product of glycogen which has been shown to undergo oxidation. The enzyme system has been discovered by Warburg and Christian (37) and has been further studied by others (38–41). Glucose-6-phosphate is oxidized to gluconic acid-6-phosphate by a specific dehydrogenase for which triphosphopyridine nucleotide is the coenzyme. By combination of the same coenzyme with other dehydrogenases a stepwise oxidative degradation of the gluconic acid molecule takes place.

In intact muscle the equilibrium ester which had accumulated as the result of various procedures (stimulation, epinephrin) disappeared three times as rapidly in O_2 as in N_2 (19). Aerobically it was partly oxidized and partly converted to glycogen; anaerobically it was converted to lactic acid (42). The conversion of the fructose part of the equilibrium ester to fructose-1-6-diphosphate is the limiting reaction for lactic acid formation in intact muscle. The path from fructose-diphosphate to lactic acid has been extensively reviewed in recent years and will not be discussed here.

Glycogen Synthesis

The following diagram illustrates various enzymatic processes in their relation to glycogen synthesis.



Enzymes: (Where no letters are given, enzymes have not been named)
(*a*) phosphorylase
(*b*) phosphoglucomutase
(*c*) isomerase
(*d*) phosphatase
(*e*) glucose-6-phosphate dehydrogenase (Zwischenferment of Warburg)

The last step in the synthesis of glycogen, namely the reaction catalyzed by phosphorylase, occurs under anaerobic conditions. The phosphorylation of glucose and fructose which is indicated by dashed arrows is dependent,

in liver and kidney, on simultaneously occurring oxidations; in other tissues this type of phosphorylation has not yet been studied. Anaerobic phosphorylation of glucose has been shown for yeast, while in mammalian tissues (perhaps with the exception of red blood corpuscles (43) and of retina (44) a clear-cut demonstration of this process is still missing.

When fructose is added to liver slices which are shaken in oxygenated Ringer's solution, a rapid conversion to glucose takes place, a reaction which does not occur under anaerobic conditions (45). When fructose-6-phosphate is added to liver slices it is split to glucose and inorganic phosphate. This involves two enzymatic reactions (*c* and *d* in the above scheme), which are not dependent on oxygen. One may conclude, that in order to be converted to glucose, fructose has to be phosphorylated, probably on carbon atom number 6 and that this initial step requires oxidative energy. Goda (46) made similar observations on liver and kidney slices and reported that phlorhizin inhibited the disappearance of fructose in concentrations in which it had little effect on respiration.

That the initial step which occurs only in oxygen and which is inhibited by phlorhizin is a phosphorylation is also indicated by the experiments of Kalckar (47). When glucose or fructose was added to kidney pulp or extract in the presence of fluoride, inorganic phosphate disappeared and fructosediphosphate was formed. These reactions occurred only in oxygen and were inhibited by phlorhizin. Liver extracts were also able to phosphorylate glucose under aerobic conditions. These observations were confirmed in our laboratory (unpublished).

The first products of phosphorylation of glucose and fructose have not been isolated, but the formation of fructose-diphosphate indicates that glucose-6-phosphate is an intermediate, as shown in the above scheme. The possibility that glucose is initially phosphorylated on carbon atom number 1 has also to be considered. While glycogen formation from glucose-6-phosphate has been shown to take place in intact muscle under aerobic conditions (42), this conversion has not as yet been obtained in tissue extracts.

In order to study the conversion of glucose-1-phosphate to glycogen in tissue extracts it is necessary to separate the phosphorylase from diastase which removes glycogen and from phosphoglucomutase and phosphatase which remove glucose-1-phosphate. Most of the diastase is removed by perfusion of the organs with Ringer's solution prior to the preparation of the extract. Adsorption with aluminum hydroxide gel, washing of the adsorbate with water, elution with sodium glycerophosphate and repeated precipitation of the eluate with 0.3 saturated ammonium sulfate separates the phosphorylase from the other two enzymes.

When glucose-1-phosphate is added to such a purified enzyme solution, inorganic phosphate is liberated and a polysaccharide is formed which may be determined according to Pflüger's method for glycogen. The values so obtained agree closely with those calculated from the inorganic phosphate liberated. Mannose-1- and galactose-1-phosphate are not converted to a polysaccharide by these enzyme solutions.

Polysaccharide synthesis has been demonstrated with phosphorylase preparations from yeast (48) and from mammalian muscle (36), heart, brain and liver (4). The polysaccharide formed by the yeast and liver enzymes gives a brown color with iodine and is indistinguishable from glycogen in all chemical properties so far investigated. Determinations of the molecular size have not yet been made. The polysaccharides formed by the muscle, heart and brain enzymes have the same properties except that the color given with iodine is pure blue with muscle, brownish-purple with heart and reddish-purple with brain. What chemical differences exist between the glycogens giving brown, purple and blue color reactions with iodine is unknown, but it may be mentioned that each of these colors has been observed before with glycogen preparations made from tissues. Claude Bernard described a blue iodine color which he obtained with glycogen extracted from denervated, previously fatigued muscle, but unfortunately he did not give the exact experimental details (49). Young (50) obtained purified muscle glycogen which gave a brownish-red to reddish-violet iodine color.

The polysaccharides prepared in the test tube from glucose-1-phosphate, when added to tissue extracts, are esterified with inorganic phosphate and converted back to glucose-1-phosphate. With the enzymes prepared from yeast and liver, reaction 1 reaches equilibrium (at 30° and pH 7) when the ratio inorganic phosphate/glucose-1-phosphate equals 5.1. With the enzymes prepared from muscle, heart and brain, the activity usually comes to an end before this value is reached. This point is still under investigation.

Another difference between the activity of enzyme preparations from liver on the one hand and from muscle, heart and brain on the other hand has been reported (4, 36). In the case of liver phosphorylase glycogen synthesis sets in immediately upon addition of glucose-1-phosphate and adenylic acid, while there is always a lag period with the muscle, heart and brain enzyme. This lag period is short (5 to 10 minutes) with comparatively crude enzyme preparations and becomes very long (2 to 12 hours) with further purification of the enzyme. This puzzling observation has now been explained. It has been noted before that enzyme preparations made from the liver of well-fed animals, even if they had been purified, contained some glycogen, while similar preparations from muscle, heart and brain were free of glycogen. It was found that addition of minute amounts of glycogen to enzyme preparations from the latter tissues promptly abolished the lag period. Concentrations of 0.005% of glycogen were still effective. This finding opens up interesting possibilities in the study of the mechanism of action of a synthesizing enzyme.

Effect of Hormones on Glycogen Metabolism

It has been clearly established in experiments on intact animals and on isolated organs that epinephrin accelerates the reaction glycogen→lactic acid in muscle and the reaction glycogen→glucose in the liver (51). In mammalian muscle and in isolated frog muscle hexosemonophosphate (equilibrium ester) increases and inorganic phosphate decreases (see page 288) which indicates that the point of action of epinephrin is reaction 1. In mam-

malian muscle in situ lactic acid production is markedly increased, so that the lactate diffuses out into the blood stream, while in isolated frog muscle lactic acid production rises only slightly. The conversion of the fructose-6-phosphate to fructose-1, 6-diphosphate, the next step in the chain of enzymatic reactions which finally leads to lactic acid, is apparently slow in frog muscle. When oxygen is supplied to isolated frog muscle, less equilibrium ester accumulates under the influence of epinephrin than under anaerobic conditions (20). This is a 'Pasteur effect,' i.e. the ester is either rapidly removed by oxidation and reconversion to glycogen, or less is formed in oxygen than in nitrogen.

Since glycogen breakdown in liver and muscle is initiated by the same enzyme, the point of action of epinephrin in these two tissues, and in other tissues, may be assumed to be the same. It is of importance to emphasize that the accelerating effect of epinephrin on phosphorylase activity is obtained in intact cells only. In enzyme solutions epinephrin has no effect on phosphorylase activity. This suggests that epinephrin exerts its effect by increasing the concentration of active enzyme in the cell. Since the mechanisms which control the rate of enzyme activity in the cell are not known, it seems hardly justified to attempt further explanations.

While insulin does not appear to be indispensable for the reaction glucose $\rightarrow$glycogen, as shown by the fact that some glycogen deposition occurs in liver and muscle of depancreatized animals at very high blood sugar levels (52), it is of importance for glycogen deposition at physiological blood sugar levels. When sufficient substrate (glucose) is supplied, an increased glycogen deposition can be demonstrated in muscle of normal animals even if the blood sugar is kept at hypoglycemic levels by administration of insulin.

In the liver marked glycogen storage has been observed in depancreatized animals following insulin injections. The apparent contradiction that insulin injections do not increase glycogen deposition in the liver of normal animals has been explained by the effect of the blood sugar level on the reaction glucose$\rightleftarrows$glycogen which will go to the left if the blood sugar is below normal, a mechanism which is essential for the regulation of the blood sugar level. Insulin (and ergotoxine) have an inhibitory effect on epinephrin glycogenolysis in liver but an analogous effect is not observed in muscle (20).

The question arises whether or not insulin influences any of the enzyme reactions discussed in this paper. The well-known decrease in the inorganic phosphate and the potassium of plasma has often been interpreted to mean an accumulation of a phosphorylated intermediate in the tissues. The analysis is complicated by the fact that insulin, because of its hypoglycemic action, causes a secondary output of epinephrin from the adrenal medulla. Epinephrin also causes a lowering of the inorganic phosphate of plasma which is, however, adequately explained by the accumulation of hexosemonophosphate (equilibrium ester) in muscle (17). When the hypoglycemic action of insulin and hence the secondary output of epinephrin is prevented by simultaneous administration of glucose, the plasma phosphates decrease, but the content of hexosemonophosphate and that of other organic phosphate fractions of muscle does not change (53). The clue to the situation is found in the fact

that glycogen deposition which occurs after a carbohydrate meal or after an insulin injection when enough carbohydrate is supplied, is associated with the retention of water in the cells. What is really retained is not distilled water as is often erroneously assumed but a salt solution of the same composition as that already present in liver and muscle cells, that is, one in which P and K are the predominant ions (54). When no glycogen deposition or an insignificant one occurs as, for example, after a carbohydrate meal in diabetic animals, no drop in blood phosphate is observed (55). Insulin when injected in fasting animals does not lead to appreciable glycogen deposition. In order to show that insulin has no or only a minimal effect on plasma P and K in such animals, it is necessary to remove the adrenals and thus avoid a secondary output of epinephrin (56).

Although there is no indication for the accumulation of a phosphorylated intermediate when glucose or glucose plus insulin is given to an animal, the possibility remains that insulin accelerates one or both of the aerobic reactions which lead to the formation of glucose-1-phosphate from glucose, as indicated in the scheme on page 291. This would bring about glycogen synthesis by the reversible enzymatic equilibrium which is catalyzed by the phosphorylase. It should be emphasized that the 'working level' of intermediary products in the intact cell is generally very low, as has been shown for yeast by MacFarlane (57). In the case of glucose-1-phosphate the ratio inorganic phosphate/glucose-1-phosphate equals 5.1 at equilibrium, so that liver or muscle would contain only 1 to 3 mg. of glucose-1-phosphate P per 100 gm. tissue, amounts which even if they were doubled would be difficult to detect with the methods available at present.

It is evident from this brief presentation that out of a multitude of facts relating to the intact animal only a few can be adequately explained in terms of chemical mechanisms. The notable advances which have been made in recent years in the study of isolated systems, particularly in the field of biological oxidations, offer some hope of unravelling the mechanism of action of insulin and of other hormones concerned with the regulation of carbohydrate metabolism.

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THE LIVER AND CARBOHYDRATE METABOLISM

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The Site of Formation of the Blood Sugar

THE SECRETION of sugar into the blood by the liver of the fasting animal was demonstrated by the brilliant pioneer work of Claude Bernard (1). The inadequate chemical and physiological methods available to him and his contemporaries initiated an involved controversy which ultimately partly obscured his original conceptions (2). It remained for F. C. Mann (3), who devised the first practical method for complete hepatectomy in mammals, to finally establish the liver as the prime factor for the maintenance of the normal blood sugar level. The presence of appreciable quantities of glycogen in the muscles of their hepatectomized dogs during profound hypoglycemia, led Mann and his coworkers (4) to conclude that muscle glycogen is incapable of sufficiently rapid conversion to glucose to play a significant rôle in maintaining the blood sugar level. This conclusion was confirmed and extended by Soskin (5) who demonstrated that the known hyperglycemic agents, epinephrin, ether anesthesia and asphyxia, have no influence whatever on the falling blood sugar level of liverless dogs. More recently Houssay and his associates have found a similar absence of the hyperglycemic action of extracts of the anterior pituitary gland, in hepatectomized toads (6, 7) and dogs (8). Soskin concluded that muscle glycogen is not an available source of blood sugar in the absence of the liver, and that the liver is the sole source of supply for the glucose in the blood in the fasting organism. He pointed out, however, that muscle glycogen might give rise to blood sugar in the intact animal by indirect means; that is, when lactic acid is produced in excess by the muscles, reaches the liver by way of the blood stream, and is there converted into glycogen. This so-called lactic acid cycle was later investigated and elaborated by Geiger (9, 10, 11), Himwich (12), Cori (13) and others.

Source Materials for the Blood Sugar

The origin of blood sugar from ingested carbohydrates or from liver glycogen resulting from carbohydrate feeding, requires only passing mention. A consideration of the non-carbohydrate precursors of blood sugar in the fed or fasting animal, necessitates the critical examination of experimental diabetes, which has been the chief medium of research in this field. It may be well to begin with the results of Minkowski (14) which initiated

this line of work, and which have too often been quoted without critical consideration.

D:N ratio. On 31 experimental days, in 9 depancreatized dogs fed on meat, Minkowski obtained D:N ratios which varied from 3.16 to 2.62, with an average of 2.8:1. The experimental days which he used to establish the average ratio were admittedly selected, since a record of all the experimental days on any single animal would show D:N ratios much higher than 2.8:1 to begin with, and ratios which fell progressively below this figure as the death of the animal was approached. The high initial D:N ratios were discarded on the basis that they represented the pouring out of preformed glycogen stores. The low D:N ratios towards the end of the experiments were disregarded because of the poor condition of the animals at that time. The reasonableness of these objections to the results of the first and last few days of each experiment cannot be denied. But a closer examination of his data makes it apparent that the experimental days were selected in a much more arbitrary manner than we have been led to believe by those who have trustingly accepted Minkowski's average as a physiological constant.

TABLE 1. THE DAYS, DURING THE DIABETIC LIFE OF HIS DOGS, WHICH MINKOWSKI USED TO COMPUTE HIS AVERAGE D:N RATIO

Dog															
	#I	—	2	3	4	5	—	—	—	9	10	—	—	—	—	—
	II	—	—	—	—	—	—	—	—	—	—	—	12	13	—	—
	III	—	—	—	—	—	—	—	—	9	10	11	—	13	—	15
	IV	—	—	—	—	—	—	—	8	9	10	—	—	—	—	—
	V	—	—	3	4	—	—	—	—	—	—	—	—	—	—	—
	VI	—	—	—	—	—	—	7	—	—	—	—	—	—	—	—
	VII	—	—	—	—	—	6	—	8	—	—	11	—	—	—	—
	VIII	—	—	—	—	5	6	7	8	—	—	—	—	—	—	—
	IX	—	—	—	—	—	—	—	—	—	—	11	12	13	14	15

Days after pancreatectomy

Thus, the selected data in any given experimental animal began as early as the 2nd day of diabetes or as late as the 12th day, and ended as early as the 4th day of diabetes or as late as the 15th day. The days reported in some experiments moreover, are not consecutive, some days being omitted for no stated reason. It must be apparent that any desired average D:N ratio might have been obtained by such arbitrary selection of experimental days, picked from experiments in which the D:N ratios fell progressively from high to low values. This criticism is supported by other of Minkowski's results, reported in the same paper but not included in the figures from which he obtained his average D:N ratio. In comparing the initial D:N ratios obtained from well-nourished and poorly nourished animals, he recorded ratios of 2.04, 2.43, 1.62, and 2.24 on the 3rd, 4th and 5th days of diabetes in the latter animals. It is difficult to understand why Minkowksi did not attempt to correlate these results with the data from which he computed his average ratio. The poor nutrition of these animals might perhaps have accounted for the failure to obtain high initial D:N ratios. But the values uniformly below 2.8:1 obtained on the days in which the approaching death of the animal was not a factor, and on days which coincided in point of time with some of the experimental days which were used to obtain his average, serve to con-

firm the entirely artificial nature of the average D:N ratio at which he arrived. These criticisms of Minkowski's work are not intended to cast aspersions on his integrity as a physiologist. Unfortunately, Minkowski, working before the days of insulin, had to deal with acutely diabetic dogs suffering from the effects of a recent anesthetic and operation. The poor condition of his animals and their rapid decline and death, undoubtedly had much to do with the nature of his results and conclusions. Nevertheless our admiration for his fundamental contributions to physiology must not blind us to the inherent defects in his work.

Pflüger (15), Embden (16) and others, subsequently reported that they had failed to obtain fixed D:N ratios at the Minkowski level. Their work was criticized on the assumed grounds of the poor condition of their animals, or of incomplete pancreatectomy. Such criticism however, cannot be leveled at the work of Macleod and Markowitz (17) who used depancreatized dogs maintained in excellent condition by the use of insulin. After the withdrawal of food and insulin from such animals (which by subsequent post-

TABLE 2. DISTRIBUTION OF D:N RATIOS DURING 138 UNSELECTED DAYS, IN 10 DEPANCREATIZED DOGS (SOSKIN)

	Range of the D:N ratio					
	Over 3.16	3.16–2.62 (Minkowski's range)	2.61–2.00	1.99–1.00	Less than 1.00	
Number of experimental days on which obtained	15	33	43	36	11	Total days 138

mortem examination were shown to be completely depancreatized) they obtained D:N ratios far below 2.8:1, after the first few days of the experiment had elapsed. Chaikoff and coworkers (18) reported similar results and found (as noted by Minkowski) that the D:N ratio was generally higher in fat than in lean dogs, and varied decidedly in the same animal, according to its nutritional condition at the time of the experiment. In 1930 Soskin (19) published a comprehensive re-investigation of the D:N ratio in depancreatized dogs, using the advanced technic made possible by the discovery of insulin. This work was done on depancreatized dogs, completely recovered from operation by the use of insulin, and presenting well-healed and non-infected wounds. The animals were maintained on a low calory, protein diet, and the absence of islet tissue was verified by post-mortem examination. In contrast to Minkowski's animals, they survived the withdrawal of insulin for as long as 5 weeks, during which they usually remained bright and active although losing weight. The observations on the D:N ratio comprised 138 unselected days in 10 dogs, in contrast to Minkowski's 31 selected days in 9 dogs. The distribution of the D:N values obtained is given in table 2.

It is seen that although some D:N ratios similar to Minkowski's were obtained, there is nothing to indicate that such values have any particular significance. In general the D:N ratio tended to be high at the beginning of each experiment and to show a progressive fall as the animals lost weight

and their stores of adipose tissue were depleted. The fact that some animals maintained D:N values far below 2.0:1 for as long as 18 days, precludes the appelation of 'premortal' which some writers have used to avoid consideration of all results which deviate from their preconceived standard.

It is clear that, if Minkowski's ratio has any meaning, the falling ratios obtained later in the experiments signify the utilization of increasing amounts of the sugar arising from protein. If, on the other hand, the low ratios represent the true extent of gluconeogenesis from protein, the Minkowski values must mean that sugar is being formed from fatty acid as well as from protein. In either case there remains no basis for concluding that sugar is derived solely from protein, or that none of the sugar so formed is utilized by the diabetic organism. It is permissible to conclude that sugar is derived partly from protein, but it is impossible to say to what extent this occurs. Similar conclusions may be drawn as regards the significance of the D:N ratio of 3.65:1 formerly obtained in so-called phlorhizin diabetes. This condition has been discussed elsewhere (19, 20) and will not be reviewed here. It is worth mentioning, however, that the fact that D:N ratios of 3.65:1 can no longer be obtained with the phlorhizin now available (21), casts even greater doubt on the validity of the intepretations which have been given to the D:N ratios obtained by the use of this drug.

We have seen that the D:N ratio does not warrant the conclusion that sugar cannot be utilized by the diabetic organism. It will be shown later that there is ample positive evidence that carbohydrate utilization is not much impaired. The dextrose component of the D:N ratio therefore represents the net remainder, when the extra-hepatic utilization is subtracted from the hepatic blood sugar formation. Once this is granted, it must inevitably be concluded that the breakdown of protein cannot supply sufficient sugar for both excretion and utilization, and that the liver forms sugar from fatty acid as well as from protein. The long controversy on this subject in the past was made possible only by the dearth of positive evidence of carbohydrate utilization and the insistence of the "non-oxidation' proponents, that all calculations be made on the assumption that none of the sugar formed by the liver was utilized in the diabetic organism. The evidence for gluconeogenesis from fat on this basis has been comprehensively reviewed by Geelmuyden (22) and Macleod (23) and will not be discussed here. It will be more profitable for us to consider the more recent evidence, in which the utilization of sugar is taken into account, or in which it is excluded as a factor by direct observations on the formation of sugar in the isolated liver.

From carbohydrate balance experiments on rats and rabbits, Cori (13) calculated the quantities of blood sugar which the liver would have to secrete in order to account for the epinephrin hyperglycemias which he observed. It was apparent that the liver could not supply the necessary amounts of blood sugar, unless the greater portion of that sugar were derived from fatty acids. Since he considered the latter process unlikely, he marshalled certain evidence from which he concluded that epinephrin hyperglycemia is chiefly due to a decreased utilization of sugar by the muscles. Soskin and coworkers (24) tested Cori's evidence and conclusions by using measurements

of blood flow to convert arteriovenous blood sugar differences into amounts of sugar retained by the muscles per unit of time. They could find no evidence that epinephrin decreased the utilization of sugar. Similar negative results were obtained when the experiments were repeated with the cooperation of Essex, Herrick and Mann (25). Still more recently, Himsworth and Scott (26) have reported that epinephrin actually increases the utilization of sugar by the peripheral tissues. Although the significance of the data which they interpreted to mean an increased sugar utilization may be open to question, their results certainly confirm the fact that epinephrin does not cause a decreased utilization of sugar. From these considerations it must be concluded that epinephrin hyperglycemia is an hepatic affair and that, if Cori's above mentioned calculations are correct, they indicate the occurrence of gluconeogenesis from fat in the liver, under the influence of epinephrin.

Recently, Young (27, 28), using figures for the sugar utilization of normal and depancreatized animals which are available from hepatectomy experiments, has calculated that sugar must be formed from fatty acids in the livers of both types of animal.

As regards the isolated liver, if the utilization of carbohydrate were not a factor in the D:N ratio of the whole diabetic animal, it would be expected that similar D:N ratios should be obtained from isolated livers (calculated from changes in the blood or perfusate) where utilization by extra-hepatic tissues is also excluded. This is not the case. Burn and Marks (29) perfused the glycogen-poor livers of fat-fed dogs. Blixenkrone-Møller (30) perfused the livers of normal and phlorhizinized cats with sodium butyrate. In both cases high D:N ratios were obtained ranging from 5.0:1 to as high as 45.0:1 for some experimental periods; similarly, Heller (31) who observed the liver in situ in the intact animal, calculated D:N ratios ranging from 5.0 to 14.0 for the livers of normal and phlorhizinized cats. It is apparent that gluconeogenesis from fat is not difficult to demonstrate when the masking effect of the simultaneous utilization of the formed carbohydrate is either taken into account, or obviated by the experimental method.

The respiratory quotient. It is becoming increasingly more evident that the respiratory quotient of the whole body, like the D:N ratio, cannot be regarded as the index of a single process. The orthodox interpretation of the non-protein R.Q. of about 0.7, which is characteristic of both the fasting and the diabetic states, involves the tacit assumption that the only vital processes (aside from protein catabolism) which are in progress and which ultimately consume oxygen and give rise to carbon dioxide, are those associated with the oxidation of fat. Yet there is very satisfactory evidence that other processes which require oxygen or yield carbon dioxide are taking place under those conditions. It is generally agreed, for example, that the brain derives its energy solely at the expense of carbohydrate, and yields an R. Q. of about 1.0 at all times (32–36). This high R. Q. must be balanced by a correspondingly low one, if the composite R. Q. of 0.7 is to mean anything at all. Indeed, this principle of different R. Q.'s forming a composite total R. Q. is generally used to interpret an R. Q. over 1.0, on the basis that the oxidative R. Q. is being elevated by a simultaneous conversion of carbohydrate into fat (37).

There is no evidence that the high R. Q. due to the latter process cannot be one of the components of any R. Q. under 1.0 (38).

In view of the composite nature of the R. Q. it is difficult to justify the orthodox interpretation of the 0.7 value, in opposition to direct evidence by chemical analysis that carbohydrate utilization proceeds under a variety of conditions in which this R. Q. is usually obtained. Such evidence is available after hepatectomy (39) or evisceration (40) of the depancreatized dog; after similar procedures on the phlorhizinized dog (20), and on the normal dog fasted to the point of so-called hunger diabetes (41); after hypophysectomy of the depancreatized dog (42, 43); and during prolonged injections of epinephrin in the normal dog (24, 25). Because of this utilization which, as pointed out above, necessitates gluconeogenesis from fatty acid as well as from protein, the diabetic R. Q. can best be interpreted as a resultant of at least two factors, namely, a low component due to the new formation of sugar from fatty acid and protein, and a high component due to the simultaneous oxidation of that carbohydrate.

Since gluconeogenesis occurs in the liver, it would be expected that R. Q. determinations performed on the isolated liver under the appropriate physiological conditions should yield very low values. This is the case. Gemmill and Holmes (44) found that the R. Q. of liver slices from a rat fed on a normal diet averaged 0.79, while that from a rat fed butter averaged 0.58. A coincident increase in the carbohydrate content of these liver slices was observed, which was greater in those from the fat fed animals. More recently, Blixenkrone-Møller (45) determined the R. Q. in the perfused livers of normal and depancreatized cats. The R. Q.'s averaged 0.57 for the normal and 0.37 for the diabetic animals. From other data, including the high D:N ratios previously mentioned, he concluded that the low R. Q. of the diabetic liver resulted from (*a*), the desaturation of fatty acids, (*b*) gluconeogenesis from protein and fatty acids (*c*) the formation of ketone bodies. We may summarize by saying that the above considerations of the respiratory quotient show that it in no way contraindicates carbohydrate utilization in the diabetic organism, or hepatic gluconeogenesis from fatty acid.

Ketosis. Like the D:N ratio and the R. Q., the ketosis in diabetes and other conditions has been explained in two chief ways. Those who accepted the Minkowski D:N and the orthodox interpretation of the R. Q. have regarded the ketone bodies as abnormal toxic products of incomplete fat oxidation, which accumulate because of the lack of simultaneously oxidizing carbohydrate (46–50). Others have found it more logical to regard the ketone bodies as essentially normal intermediate metabolites, appearing during the conversion of fatty acid to sugar, and accumulating in abnormal amounts whenever the rate of gluconeogenesis from fat becomes excessive (22, 23, 51, 19). Parenthetically, it should be mentioned that it is agreed that unimportant amounts of ketone bodies may also arise from certain amino acids. Beginning with Embden and coworkers (52) many investigators demonstrated that the liver was the chief source of the ketone bodies, and that the fatty liver forms more ketone bodies than one rich in glycogen (53, 54). It was also found that livers from depancreatized or phlorhizinized animals produced

more ketone bodies than livers from normal animals (55). But the crucial difference between the two viewpoints outlined above, was that the first theory ascribed the ketosis of diabetes to an inability of the extra-hepatic tissues to dispose of the ketone bodies, while the second hypothesis postulated a rate of hepatic formation in excess of the existing normal ability for peripheral disposal. The work of Chaikoff and Soskin (56) strongly supported the latter viewpoint. They showed that the tissues of the eviscerated depancreatized dog disposed of injected sodium acetoacetate with equal facility as compared to the extra-hepatic tissues of the normal dog, and that the apparent slower rate of disposal after injection into the intact diabetic animal, was due to the excessive amounts of endogenous ketones with which the tissues of the animal were already dealing. More recently, the same viewpoint has been firmly established by the outstanding work of Mirsky and collaborators. Using a ketogenic extract of the anterior pituitary gland, they demonstrated that the ketones so formed arise only in the liver (57), that insulin which suppresses this ketosis does so by acting on the liver and not on the extra-hepatic tissues (58), and that the rate of disposal of the administered sodium salt of β-hydroxybutyric acid by the extra-hepatic tissues was independent of the amounts of sugar being utilized by those tissues (59, 60, 61). This and other evidence make it no longer possible to regard ketosis as indicating a lack of carbohydrate oxidation by the muscles. It is clear that in diabetes the ketone bodies, as well as the blood sugar, are utilized by the extra-hepatic tissues, but are produced by the liver at rates which exceed the power of the tissues to dispose of them. Indeed, we now have some direct evidence that the ketone bodies may be intermediary metabolites in the overproduction of sugar from fatty acids, for Weil-Malherbe (62) has very recently demonstrated the in vitro formation of sugar from acetoacetic acid by kidney slices.

Hepatic Regulation of the Blood Sugar

The relative constancy of the blood sugar level in the normal animal, under varied conditions of feeding and fasting, indicates the existence of adequate regulating mechanisms. On the other hand, the hyperglycemia and the great dependence of the blood sugar level of the diabetic organism on the kind and amount of ingested food, suggests some disturbance in the regulating mechanisms. Claude Bernard realized that the normal blood sugar level represented a precise equilibrium between the rates of sugar formation in the liver and sugar utilization in the tissues (2). While his conception has been confirmed by most recent workers (63–70) it has nevertheless been virtually ignored in the explanation of the various experimental or clinical states which are characterized by a persistence of abnormal blood sugar levels. Thus the rapid and accurate adjustment which is represented by the normal dextrose tolerance curve has been generally explained in terms of increased sugar utilization and storage, resulting from increased insulin secretion (13, 71–78). Conversely, the diabetic type of dextrose tolerance curve, whether occurring in experimental or clinical diabetes, starvation, or toxemia, has been attributed to a lack of pancreatic response to the ad-

ministered sugar and a consequent deficiency in sugar utilization and storage (71, 76, 78, 79, 80, 81, 82). However, Soskin and coworkers (83) have recently shown that completely depancreatized dogs, receiving a constant injection of insulin sufficient to maintain a constant normal blood sugar level, yield normal dextrose tolerance tests, including the Staub-Traugott phenomenon and the hypoglycemic phase (84). But hepatectomized dogs,

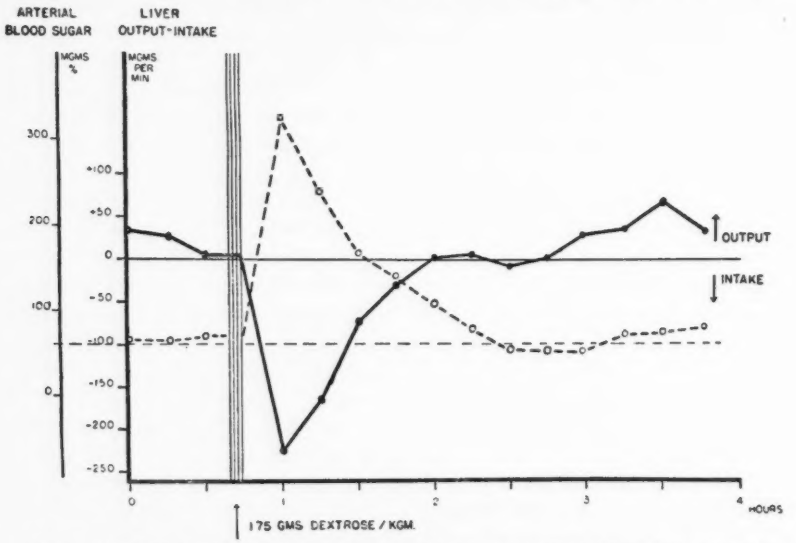

Fig. 1. The effect of dextrose administration upon the output and intake of sugar by the liver of an intact dog, calculated from blood sugar values and thermostromuhr measurements of hepatic bloodflow (85). The broken line represents arterial blood sugar values; the heavier continuous line represents output or intake of sugar by the liver in mg. per minute. Note the immediate cessation of sugar output when sugar is administered, and the large intake of sugar which follows. Throughout the 2nd hour after sugar administration the liver neither retains nor excretes sugar. During this period the level of sugar in the arterial blood falls below its original control values, and does not return to normal until after the liver has resumed its output. The inhibition of the hepatic secretion of sugar is, therefore, a real and separate phenomenon from the storage of sugar.

with pancreas intact, receiving a constant injection of dextrose sufficient to maintain a normal blood sugar level, invariably yield markedly diabetic tolerance curves. It was concluded that the pancreas is not essential to the metabolic reactions which determine the normal dextrose tolerance curve, while the presence of the normal liver is essential. From this and other data, it was postulated that, in the presence of a sufficiency of insulin, but not necessarily an extra secretion from the pancreas, the normal liver, as one of its responses to administered dextrose, decreases the output of blood sugar which it had been previously supplying from its own resources. This homeostatic mechanism was directly proven by Soskin, Essex, Herrick and Mann (85), using blood sugar values and thermostromuhr measurements of bloodflow to calculate the quantitative output and intake of sugar by the intact liver in situ. It should not be implied that this hepatic mechanism is the only one involved in dextrose tolerance, for glycogen deposition in liver and

muscle undoubtedly play a part and, as Soskin and coworkers (40) have recently shown, the rate of sugar utilization varies directly with the height of the blood sugar level in both normal and depancreatized animals. However, the diabetic type of tolerance curve obtained in the hepatectomized animal, certainly indicates the major importance of the liver in this regard. It is time, therefore, to apply the now substantiated fundamental conception of

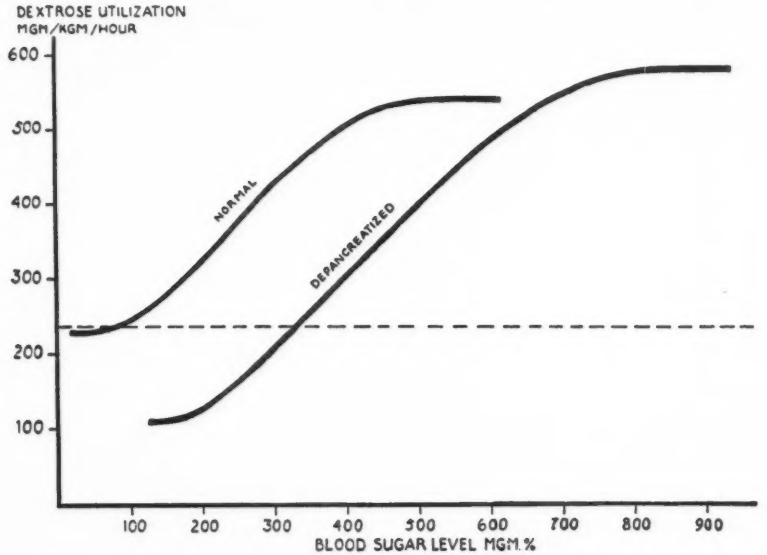

Fig. 2. The relation between the blood sugar level and the rate of sugar utilization in the normal and diabetic organism, calculated from carbohydrate balance experiments in eviscerated normal and depancreatized dogs (40). The heavy continuous lines are smooth curves drawn through the scatter of points plotted for each group of animals. In both types of animal the rate of sugar utilization varies directly with the blood sugar level. The diabetic animal uses less sugar than the normal at any given blood sugar level. But the lighter broken line, which marks the rate of sugar utilization in normal animals at the normal blood sugar level, crosses the diabetic curve at somewhat over 300 mg. %; showing that the diabetic animal at the hyperglycemic level which it ordinarily maintains, uses as much sugar as the normal animal at the normal glycemic level.

Claude Bernard, to the explanation of the various phenomena associated with disturbances of the glycemic level.

In the normal animal, a rise in the blood sugar level causes the liver to respond by diminishing its output of sugar to the blood. A coincident decrease in gluconeogenesis, by removing a low component of the composite R. Q., can explain part of the rise in the R. Q. which follows sugar administration. The stimulus which elicits the hepatic inhibitory response is the blood sugar itself, and the threshold of stimulation of the hepatic mechanism in a particular animal depends largely upon the endocrine balance, and coincides with the level of blood sugar which that animal habitually maintains. Although other glands are undoubtedly involved, it may be supposed for the sake of simplicity that the endocrine balance consists of the opposing influences of the hormones of the pancreas and of the anterior hypophysis.

When insulin is lacking or when anterior pituitary hormone is present in excess, the liver threshold rises and hyperglycemia results. An excess of insulin or a deficiency of the anterior pituitary hormone lowers the liver threshold so that hypoglycemia occurs. When both insulin and the anterior hypophysis are lacking, as in the 'Houssay' animal, the diabetes is ameliorated, and it is possible to obtain most of the criteria of normal carbohydrate metabolism (42, 43) including normal dextrose tolerance curves at a glycemic level in between the usual blood sugar levels of hypophysectomized and depan-

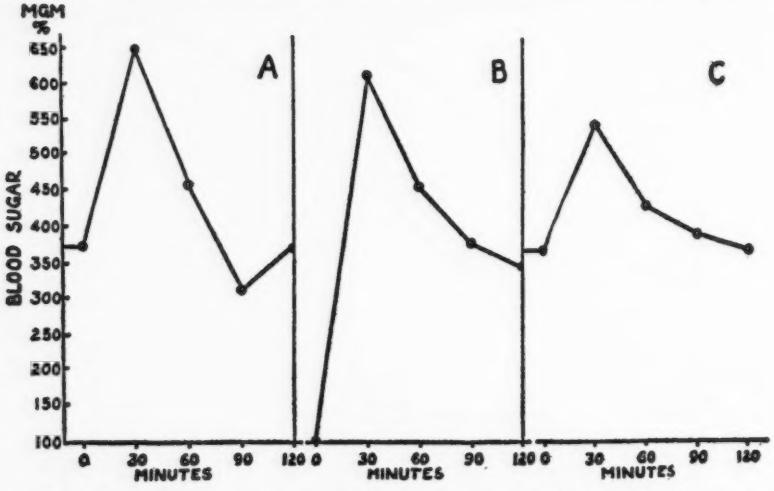

Fig. 3. Dextrose tolerance curves obtained at different times in the same hypophysectomized-depancreatized dog (86), showing the persistence of the blood sugar regulating mechanism of the liver. The threshold of stimulation of this mechanism is elevated, but not to the infinite height to which it is raised in animals with only the pancreas removed. The initial control blood sugars for curves A and C were at the level usually maintained by this animal in the post-absorptive state, when food intake was ample. Prior to the experiment in which curve B was obtained, the animal had been fasted until the blood sugar level fell to 100 mg. %. It may be seen that, except for the initial rapid rise of the blood sugar in curve B, all 3 curves are strikingly similar. It is evident that the 'diabetic' appearance of curve B is due to the fact that the threshold for the homeostatic mechanism did not vary with the initial blood sugar level, but remained fixed by the experimentally altered endocrine balance. Thus the administered sugar in curve B caused an unchecked rise in blood sugar until the threshold value was reached. As soon as this was attained, the normal response of the liver occurred.

creatized animals (86). In the depancreatized animal with hypophysis intact, the hyperglycemias caused by sugar administration do not reach the infinitely high threshold of the diabetic liver, which therefore is not inhibited and continues to form and pour sugar into the blood. This lack of hepatic inhibitory effect accounts for the difficulty in influencing the diabetic R. Q. and for the so-called quantitative return of the administered sugar. Thus the metabolic disturbance in diabetes regarded from the standpoint of blood sugar regulation, coincides with the 'overproduction theory' which we have already been forced to adopt on the basis of the other evidence which we have evaluated. From the same standpoint, other disturbances of carbohydrate metabolism such as those occurring in toxemia (87, 88) are also readily understood.

In conclusion, it must be emphasized that all of the foregoing discussion depends upon a simple but fundamental concept, namely, that carbohydrate metabolism represents a dynamic balance between blood sugar formation in the liver and its utilization by the body tissues. Any disturbance in carbohydrate metabolism is likely to affect both sides of the equilibrium, whether directly or by secondary compensatory changes. All problems must therefore be considered from both aspects. Since we are dealing with opposing forces, where a positive change in one may be equivalent to a negative change in the other, it is usually necessary to study each separately while the other is experimentally excluded, in order to judge the extent to which each participates in the final equation. This we have attempted to do.

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THE ADRENAL CORTEX AND CARBOHYDRATE METABOLISM

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A DIRECT PARTICIPATION of the adrenal cortex in the hormonal control of carbohydrate metabolism has been the subject of vigorous debate for a number of years. The interest in this problem has centered not only on the interpretation of the alterations in carbohydrate metabolism that occur in adrenalectomized animals, but also on their relation to the function of the adrenal cortex. This problem would appear to be capable of a simple solution were it not for the fact that other changes follow adrenalectomy that at first sight appear to be of greater significance than the disturbances in carbohydrate metabolism that have been reported.

There is now no doubt that many adrenalectomized animals suffer such widespread disturbances in their water and electrolyte balance that their ultimate death can hardly fail to be associated with them. This is the view taken by such investigators as Loeb (1), Harrop (2), Zwemer (3), Swingle (4), Kendall (5) and Hartman (6), although it should be pointed out that there is no general agreement among these workers as to the exact mechanism by which these alterations in metabolism are induced by adrenalectomy. Their results have, however, led to the general conclusion that the intimate function of the cortical hormone is to be found in the influence it exerts over some phase of electrolyte and water metabolism, regardless as to whether the site of its action is primarily in the kidney or elsewhere, or whether it exerts a specific influence on sodium or potassium metabolism.

It would be fair to say that none of those supporting the above view denies that alterations in other phases of metabolism are to be observed in adrenalectomized animals, but they appear to regard these as a consequence of the general condition of the organism after adrenalectomy rather than furnishing any indication of the function of the adrenal cortex. Among alterations of this character has been placed disordered carbohydrate metabolism.

Such a view, however, has not met with general acceptance. Britton and his colleagues (7–14) have argued that the observed alterations in carbohydrate metabolism are of such a kind and occur to such an extent that it is equally logical to regard them both as the cause of death of adrenalectomized animals and as an indication of the 'prepotent' function of this gland.

The details of their work will be discussed later, but if the alterations in carbohydrate metabolism they report are a direct consequence of adrenalec-

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tomy, then some explanation of cortical function must be found that links these findings with the observed disorders in electrolyte and water metabolism. Such an all-inclusive theory has not yet been forthcoming, but since loss of the same hormone is apparently responsible for all the effects observed, any theory of adrenal cortical function must take both these sets of facts into consideration.

The present paper is concerned with the experimental evidence that we consider justifies the belief that the hormone of the adrenal cortex plays a direct and positive rôle in the control not only of the metabolism of carbohydrates, but also that of proteins.

I. THE CARBOHYDRATE LEVELS OF ADRENALECTOMIZED MICE AND RATS

The occurrence of decreased carbohydrate levels in the blood and tissues, particularly the liver, of adrenalectomized animals has been extensively studied without any universal agreement as to its extent or frequency, and with still wider disagreement as to its significance when analysis has shown it to be present.

In 1909, Porges (15) reported that patients suffering from Addison's disease had a low blood sugar. This has often since been confirmed, very recently by Norris (16). It has, however, been denied as well by some clinicians (17). Porges carried out a few experiments with dogs in which he extirpated both adrenals. He found that the blood sugar fell rapidly after the operation and had reached the low value of 55 mg.% in about 3 hours. This result was also seen by Bierry and Malloizel (18) who are usually given credit for having been the first to observe this phenomenon.

Since this early work, the technic of adrenalectomy has considerably improved but the results of studies of the blood sugar have varied from laboratory to laboratory. Cori and Cori (19) found values below normal in their rats, as did Wyman and Walker (20). This was not confirmed by Artundo (21). In all three instances the animals survived the operation for many days and it seems quite probable that the animals in the two former instances showed the effects of what Wyman and Walker termed 'chronic insufficiency.' In experiments reported in which there seemed to be no remnant of adrenal tissue left, low blood sugars have been noted. Thus, Zwemer and his coworkers (22, 23) found that in the cat the symptoms of adrenal insufficiency were accompanied by a fall in blood sugar. They also studied adrenal demedullated cats but found no change in the blood sugar. Normal values were also noted after removal of one adrenal. Bøggild (24) observed the same effect in the dog. When one adrenal was removed and the other demedullated, the blood sugar remained within normal limits. When both adrenals were removed (rats), the blood sugar fell to levels between 18 and 64 mg.%. In a long series of extensive studies on many species, Britton and his collaborators (7–14) have always emphasized the decline in the blood sugar level which occurs after adrenalectomy. Thaddea (25) has confirmed their results.

Along with many other problems related to the adrenals, there is no universal agreement on this point. In 1927, Swingle (26) reported that in cats convulsive levels of blood glucose occur after adrenalectomy. These low values followed the development of lethargy and muscular asthenia. In 1936, however, he reported (27) that the withdrawal of cortical extract from previously adrenalectomized dogs did not always result in a fall in blood sugar. If the operation was performed in one stage, the animal

sometimes showed a decline in blood glucose which was explained as probably due to muscular traumatization. Harrop and Weinstein (28) found the values very inconstant, depending upon the condition of the dog and the time of previous feeding, while others (29) have felt that the blood sugar decreases only in the terminal stages, at which time a moderate diminution occurs. Hartman et al. (30) agree that any marked changes are manifest only during the last part of the animal's existence. Hartman, Brownell and Lockwood (31) observed that adrenalectomized rats were unable to maintain their blood sugar at normal levels when exposed to low temperatures. This entire latter group of workers have either believed that the changes in blood sugar were only terminal conditions or were the result of other disturbances that in themselves materially shortened the existence of the animal. Unfortunately, in many instances this is a just criticism. Recent studies have, however, been more carefully conducted and persistent reports of blood sugar changes cannot be so easily rejected.

If the primary disturbance is in carbohydrate metabolism, the question might well be raised as to whether the administration of glucose will prolong the life of the adrenalectomized animal. Such is not the case, for although glucose may temporarily alleviate the symptoms of adrenal insufficiency, no permanent benefit results and, indeed, animals thus treated may die with high levels of blood glucose. Zwemer (32) fed glucose daily to adrenalectomized cats and was able to prolong their lives to an average of 9 days as compared with 60 hours in the control group. The onset of symptoms was also reported to be much more gradual.

This difference of opinion on the significance and occurrence of hypoglycemia in adrenalectomized animals is duplicated in the results of other studies that have been made on the behavior of liver and muscle glycogen after adrenalectomy. Even when allowance is made for the inadequate technics used by many of the earlier workers in obtaining samples of liver and muscle glycogen for analysis, and in too many instances this also applies to more recent work, there is still to be found marked divergence in the results from different laboratories.

Porges (33) first reported the occurrence of very low liver and muscle glycogen values a few hours after he had extirpated the adrenal glands. His dogs had been fasted for 24 hours before operation. When he fed them glucose a few hours beforehand, he found considerable amounts of glycogen still present at the time he examined the tissues, 4½ hours after adrenalectomy. Schwarz (34) confirmed this work, as did Kahn (35). Probably incomplete removal of the adrenals occurred in the experimental animals used by the latter workers who reported that some of their rabbits survived for a year. It does not seem of value to elaborate further on many other reports on the occurrence of low glycogen levels in adrenalectomized animals inasmuch as the conditions under which they were obtained render them of doubtful importance.

In 1927, Cori and Cori (19), in their careful work were impressed by the fact that after adrenalectomy, rats which had been fasted 24 hours had practically no liver glycogen. Unfortunately, some of their rats probably retained adrenal cortical tissue as judged by their extended survival, but the results were nevertheless valid because the animals were probably in a state of 'chronic insufficiency.' It is important to note that these were fasted animals.

Recently, Britton and his coworkers (7–14), in their studies have claimed that muscle and liver glycogen are markedly reduced in many species of animals after adrenalectomy. The figures they present from their studies on muscular tissue show a considerable range of values. Leloir (36) found low liver glycogen in dogs and a slight lowering of muscle glycogen. There is a wide range in the latter values and a statistical treatment of the data would doubtless not substantiate his claim. Thaddea (25)

made similar observations. His data show, for example, in one instance 316 mg.% of muscle glycogen in the tibialis before operation and 148 mg.% after, values which are probably too low because of glycogenolysis.

The conditions under which a solution of this problem may be attempted require the recognition of certain complicating factors that are encountered in dealing with adrenalectomized animals. The first of these is the question of the food ingested prior to the glycogen analyses. In some species, particularly the cat, all food may be refused for several days before death, while in others food may be eaten up to a few hours before it occurs. Obviously, this difference will greatly influence the levels of carbohydrate in the tissues when the examination is made. Arising from this is a second problem. Britton and his colleagues have recently reaffirmed (37) that not only do adrenalectomized animals of several species exhibit reduced carbohydrate levels, but they also show a much decreased ability to form liver and muscle glycogen from ingested carbohydrate. This claim is contrary to the reports of Kuriyama (38), Cori and Cori (19), Deuel et al. (39). All these workers, however, used only rats or mice.

Another consideration is the rapidity with which serious alterations in the electrolyte balance occur after adrenalectomy. In some species these seem to cripple the animal more rapidly than in others, and hence, it may be argued that death occurs before the carbohydrate levels have declined to any great extent.

Carbohydrate levels. In the present series of experiments, we have used adequate technics to obtain tissue samples for glycogen analysis and have confined our observations to the rat and mouse, since in these species not only life but a practically normal water and electrolyte balance may be maintained by the judicious use of sodium salts. We have studied adrenalectomized animals maintained in this manner both in the fed and fasted state.

The results, which are summarized in table 1 indicate that so long as the animals are fed, normal levels of liver and muscle glycogen are found, but that on subjection to fasting there is a rapid decline in the liver glycogen which is ultimately followed by a depletion of the muscle glycogen. In addition, at least in the rat, there occurs a well-marked hypoglycemia which is particularly notable after a fast of 48 hours. At this time hypoglycemic convulsions are not infrequent and require glucose administration for their relief. It is noteworthy that following glucose administration and realimentation, the animals remain well, a result that is not seen in those that have not received sodium salts. In this instance, glucose affords only a transitory relief and death soon follows.

It may be concluded that the observations of Britton and his colleagues on the depletion of the carbohydrate levels of adrenalectomized animals are correct, insofar as fasted animals are concerned, but that we cannot confirm the observation that fed animals exhibit any noteworthy deviations from the normal. The reason for the last discrepancy seems to lie in the condition of the water and electrolyte metabolism of the animals at the time glucose is administered. It does not seem unreasonable to assume that if these are markedly disorganized, the ability of the tissues to store glycogen is also impaired. Such a consequence might well be encountered in any condition in which the circulation and blood pressure were depressed to the degree commonly found in the later stages of adrenal insufficiency.

TABLE 1. CARBOHYDRATE LEVELS OF NORMAL AND ADRENALECTOMIZED MICE AND RATS

No. of animals	Length of fast, hours	Glycogen, mg. %		Blood glucose, mg. %	Condition
		Liver	Muscles		
		A. Mice			
11	0	2840±400	435±54	—	Normal
12	0	2177±284	479±19	—	Adrenx.
5	6	171± 85	332±52	—	Normal
7	6	86± 30	238±33	—	Adrenx.
8	12	252± 92	298±19	—	Normal
5	12	102± 51	269±16	—	Adrenx.
8	24	346± 21	228±11	—	Normal
4	24	44± 14	158±30	—	Adrenx.
		B. Rats			
10[1]	0	1780±370	590±25	124±3	Normal
7[1]	0	2310±600	533±29	97±4	Adrenx.
10[1]	24	25± 1	520± 8	72±2	Normal
13[1]	24	16± 1	432±14	54±4	Adrenx.
7	48	233± 13	507±23	80±3	Normal
4	48	70± 1	358±21	30±2	Adrenx.

[1] These figures are taken from recent results of Dr. J. A. Russell.

Nitrogen excretion. Evans (40) found that fasted adrenalectomized rats excreted about 25% less nitrogen than did normal rats under similar conditions. This result has recently been confirmed by Harrison and Long (41). Since, as will be seen later, the administration of an excess of cortical extract to normal fasted rats results in an increased nitrogen excretion and a new formation of carbohydrate, the diminution in protein catabolism observed in adrenalectomized rats is probably a direct consequence of the loss of the cortical tissue. Furthermore, since the bulk of the evidence indicates that the blood glucose during fasting is maintained by the protein catabolism, the occurrence of hypoglycemia in fasted animals after adrenalectomy becomes explicable on these grounds.

II. THE EFFECT OF CORTICAL EXTRACT ON METABOLISM

The alterations in carbohydrate metabolism following the administration of active extracts of the adrenal cortex were first studied extensively by Britton and Silvette and reported in a series of papers (7–14). Since 1931, they have performed a large series of experiments on the effects of cortical extract on the carbohydrate levels of many species of animals. In all their work, they have found that injections of potent extracts result in a rise in blood sugar and liver and muscle glycogen in normal and adrenalectomized animals. They found that the low blood sugars which they encountered after adrenalectomy could be prevented or restored to normal by suitable treatment with extracts. Similarly they reported that it was possible to abolish the loss of muscle and liver glycogen or to re-establish normal levels if these were first allowed to decline. Glucose treatment produced a high blood sugar but did not fully

restore the glycogen content of the liver and muscles. Adrenalin brought about no improvement in the carbohydrate stores. In normal animals, they claimed similar effects. After injections of extracts either in single or multiple doses, rats were found to have higher blood sugar and glycogen levels than saline-treated or epinephrine-treated controls. In some cases, the blood sugar rose to a point twice as high as the normal value.

Leloir (36) has confirmed the rise in blood sugar after injections of cortical extract into adrenalectomized rats, but was unable to substantiate the effect on the glycogen stores. He employed rather small amounts of extract compared to those used by Britton. He found, however, that the hormone enabled the fatigued muscle of adrenalectomized rats to restore the glycogen to normal or even supernormal levels following fatigue by a tetanizing current. This is in contrast to the failure of muscle glycogen resynthesis in untreated adrenalectomized animals. The data presented for muscle glycogen show a considerable deviation from the mean and, in many instances, are considerably lower than other values found in the literature.

Thaddea (25) has confirmed Britton's results in all respects. Zwemer and Sullivan (22) reported marked increases in blood glucose after injection of cortin. They found a slow, prolonged increase which they believed was not due to epinephrin, although they admitted their preparations contained some of this substance. They found that epinephrin brought a rapid increase of short duration in contrast to the slow, steady rise after cortin.

Regnier and Simonnet (42) injected an alcoholic extract of the adrenal cortex into normal and adrenalectomized dogs and found a rise in blood sugar to almost twice the normal level which persisted for 24 hours.

Experiments in dogs, however, have not always led to similar conclusions. Hartman et al. (30) found in the late stages of adrenal insufficiency a low blood sugar which returned to normal after treatment with a potent extract. Parkins, Hays and Swingle (27) found no change in blood sugar in an operated dog which received 3 cc. of cortical extract per kg. of body weight. Swingle and his associates have held to the opinion that cortin does not raise the blood glucose. The injection of what was deemed large quantities of extract produced no changes in blood sugar (Harrop and Weinstein, 28). Amounts up to 1800 dog units were employed.

In man, adrenal cortical tumors occasionally appear. Doubtless, many of these are liberating large amounts of the cortical hormone. In a review of 55 cases of hyperplasia and tumors of the adrenal cortex, Lukens, Flippen and Thigpen (43) found that nearly half (44%) showed glycosuria. Seven of a total of 11 instances of hyperplasia of the adrenal cortex showed glycosuria. The case which they reported showed a typical diabetic glucose tolerance curve and glycosuria.

Carbohydrate levels. We have carried out a large number of experiments on both normal and adrenalectomized mice and rats, taking what we regard as adequate precautions to maintain the electrolyte balance by the use of sodium salts. The response of the carbohydrate levels to the injection of cortical extract has been followed both in the fed and fasted state and since Britton has found that higher levels of glycogen are produced by a series of injections rather than by one large dose, this procedure has been followed in the present series of experiments.

a). Mice. Except as otherwise noted, all animals were fasted previous to the injections of cortical extract, in order to deplete the livers of glycogen and to bring the muscle glycogen to the basal level. Table 2 shows a series of

mice which had been fasted for 12 hours and were then injected with 0.25 cc. of Upjohn's cortical extract every hour for 10 to 12 hours. At the end of this time, the animals were anesthetized with nembutal, and liver and muscle were taken for analysis. The data show that after such treatment there is present 10 to 20 times the amount of liver glycogen found in normal controls injected with 0.25 cc. saline per hour for 10 to 12 hours. Inasmuch as the liver glycogen is already depleted at the end of the 12-hour fast (table 1), this must mean that a new formation of glycogen from non-carbohydrate sources has taken place. In a control series of mice, periodic injections of epinephrin in the amount present in the cortical extract (1:1,000,000) had no effect on liver glycogen.

TABLE 2. EFFECT OF CORTICAL EXTRACT, CORTICOSTERONE AND DEHYDROCORTICOSTERONE ON THE LIVER AND MUSCLE GLYCOGEN OF NORMAL AND ADRENALECTOMIZED MICE

No. of animals	Period of injection, hours	Total fast, hours	Glycogen (mg. %)		Notes
			Liver	Muscle	
			A. Normal Mice		
8	0	24	346±212	288±11	Fasted controls
7	10–12	24	2986±490	223±34	Fasted+C.E.[1]
10	8–10	24	1890±163	—	Fasted+corticosterone[2]
6	8	24	2260±263	—	Fasted+dehydrocorticosterone[2]
4	8	24	315± 84	—	Fasted+epinephrin (1:1 million)[1]
11	0	0	2840±400	435±54	Fed controls
24	8–24	0	9196±270	1014±84	Fed+C.E.[1]
			B. Adrenalectomized Mice		
4	0	24	44± 14	158±30	Fasted controls
5	12	24	2370±231	182±50	

[1] 0.25 cc. cortical extract every hour.
[2] 0.1–0.2 cc. every hour. 1 cc. contains 0.25 mg.

It is important to note that no change in muscle glycogen could be detected after the treatment with the cortical extract. This is not in agreement with Britton's results. He found that the level of muscle glycogen, as well as of liver glycogen, was raised by the action of adrenal cortical extract. However, his determinations were made after the animals had been killed by a blow on the head, and this may account for the difference in results.

In a small group of mice in which blood was taken for analysis, a higher level of blood glucose was found in the group treated with adrenal cortical extract than in 2 control animals injected with saline. The injected group of 5 mice showed an average of 100 mg. % of blood sugar as compared with 2 control mice averaging 81 mg. %.

In the fed mouse, very unusual effects of adrenal cortical hormone were observed. These animals were allowed free access to food during the period of injection. When they were killed at the end of the experiment, they had accumulated large amounts of glycogen in the liver. An average level of 9196 mg. % was observed, and in some cases nearly 12 % of the weight of the liver was made up of glycogen. So far as could be ascertained, these are the highest

values for liver glycogen reported in this species. In these animals there was observed a rise in the level of muscle glycogen which was found to average 1014 mg. %.

Throught the kindness of Dr. E. C. Kendall, there was available for these experiments a small amount of the active crystalline compounds corticosterone and dehydrocorticosterone which he had isolated from the adrenal cortex. These were injected into mice in the same manner as the cortical extract. Both these compounds were able to bring about increases in liver glycogen similar to those produced by cortical extract (table 2). There was no significant difference between the two compounds in this respect. As Kendall had already found that administration of these compounds would indefinitely prolong the life of adrenalectomized animals, it is of importance to find that they also influence carbohydrate metabolism.

As has already been pointed out, adrenalectomized mice show an almost complete disappearance of liver glycogen when subjected to a short fast. Presumably this is due to the absence of the cortical hormone. If this is true, it should be possible to restore the carbohydrate metabolism of the adrenalectomized animal to normal by treatment with adrenal cortical hormone.

Adrenalectomized mice were fasted for a period of 12 hours and then injected for 12 hours with 0.25 cc. of Upjohn's cortical extract every hour. When they were killed at the end of this time, they had considerable quantities of glycogen present in their livers, amounting to nearly as much as had been found in the normal injected mice (table 2). As before, there was no increase in the muscle glycogen. Thus, it was possible in fasting adrenalectomized mice not only to prevent the disappearance of liver glycogen, but to bring about an accumulation of considerable amounts of it by treatment with cortical extract.

b). *Rats.* When normal 24 or 48-hour fasted rats were injected with 1 cc. of Upjohn's cortical extract every hour during a further 8 to 12 hours of fasting, they, too, showed an increase in liver glycogen. The liver glycogen did not reach such high levels as in the mouse, but there was usually a five- to ten-fold increase over that of untreated rats. No change could be detected in the muscle glycogen but a 30% rise in blood sugar occurred (table 3).

These findings are in agreement with those of Britton and Silvette in the case of liver glycogen and blood glucose. However, the present studies show that the amount of muscle glycogen is almost exactly the same in the untreated as in the treated animals. In this respect, Britton and Silvette's findings are not confirmed. As has been mentioned above, the difference is probably due to the technic employed in obtaining the tissues for analysis.

In another respect the experiments of Britton and Silvette differ from those reported here. Their animals were not subjected to a preliminary fast before being treated with cortical extract. Instead, food was removed before the first injection. The presence of glycogen in the liver at the end of the experiment might, therefore, be taken as an indication that the cortical extract was important merely in maintaining the glycogen already present in the liver. In the present experiments, however, stored liver glycogen was depleted

by a preliminary fast of 24 hours or longer, thus clearly indicating that gluconeogenesis had occurred.

As in the case of the adrenalectomized mice, the injection of cortical extract into adrenalectomized rats brought about an accumulation of liver glycogen. These animals were subjected to a fast of at least 12 hours and, in most cases, 24 to 36 hours before beginning the injection of 1 cc. of cortical extract per hour for 10 to 12 hours. The data in table 3 show that at the end of this time there was almost as much liver glycogen in these operated animals as in the normal treated rats. The blood sugar was raised to a slightly higher level than that ordinarily found in normal fasted rats. This was in contrast to the low blood sugar encountered in saline treated, fasting adrenalectomized control rats. Just as in the previous experiments, the muscle glycogen was not

TABLE 3. EFFECT OF CORTICAL EXTRACT ON THE CARBOHYDRATE LEVELS OF FASTED NORMAL AND ADRENALECTOMIZED RATS

No. of animals	Period of injection, hours	Length of fast, hours	Total cortical extract cc.	Glycogen, mg. %		Blood glucose, mg. %	Notes
				Liver	Muscles		
A. Normal Rats							
5	0	24	0	206± 6	508±29	63±6	Controls
7	0	48	0	233± 13	507±23	80±3	Controls
12	8	32	8	848± 30	521±23	95±1	1 cc. C.E./hour
14	12	36–54	12	1644± 48	536±18	95±2	1 cc. C.E./hour
B. Adrenalectomized Rats							
13	0	24	0	16± 1	432±14	54±2	Controls
4	0	48	0	70± 1	358±21	30±2	Controls
4	11	35	11	652±170	421± 9		1 cc. C.E./hour
6	12	48	12	1778±430	411±28	93±3	1 cc. C.E./hour

altered by the treatment with cortical extract. Although it had not fallen to the level found in untreated rats, it was below the normal level of muscle glycogen. This again emphasizes that the effect of the cortical hormone on carbohydrate metabolism is primarily manifest by the change in liver glycogen and blood glucose.

The source of the carbohydrate formed. The formation of liver glycogen and blood glucose under the influence of cortical extract raises the question as to the source of this new carbohydrate. Inasmuch as the muscle glycogen remains constant, the possibility of a shift in the distribution of tissue carbohydrate is eliminated. Since the transformation of fat to carbohydrate has never been conclusively demonstrated, the obvious source would seem to be the tissue protein.

For this reason a study of the nitrogen excretion of both normal and adrenalectomized rats injected with cortical extract was undertaken. The animals were fasted for approximately 24 hours, and were then placed in metabolism cages and the urine was collected for 12 hours. During this time the rats were injected with 12 cc. of 0.9% sodium chloride solution. At the end of 12 hours, the cages were washed with several small portions of distilled water

which was added to the urine. Injection of the animals with 1 cc. of cortical extract was then begun and continued every hour for the next 12 hours. In this manner each animal acted as its own control, and the nitrogen excreted during the period of injection could be compared directly with the previous control period. In addition, urine was collected from control animals during two corresponding 12-hour periods in order to have parallel controls as well. The results have been summarized in table 4.

It will be seen that the increase in liver glycogen and blood glucose is accompanied by a parallel rise in the urinary nitrogen.

From the liver and body weights of the animals and the analytical data, the total amount of carbohydrate in the body was determined in both the injected and the control groups. The carbohydrate levels in the controls were calculated as milligrams of glycogen or glucose per 100 gm. of rat. The livers of the untreated group contained an average of 7 mg. of glycogen compared

TABLE 4. EFFECT OF CORTICAL EXTRACT (1 CC. AN HOUR FOR 12 HOURS) ON CARBOHYDRATE LEVELS AND NITROGEN EXCRETION OF FASTED NORMAL, ADRENALECTOMIZED AND HYPOPHYSECTOMIZED RATS.

All values are expressed as mg. per 100 gm. of body weight. The muscle weight is taken as 50% of the body weight and the glucose as distributed in 50% of the body weight.

	Normal Rats			Adrenalectomized Rats			Hypophysectomized Rats		
	Control (12)	Injected (6)	Increase	Control (4)	Injected (4)	Increase	Control (6)	Injected (11)	Increase
Liver glycogen	7±1	79±7	72	2±0.4	80±8	78	3±0.1	26±5	23
Muscle glycogen	253±15	260±15	N.S.[1]	179±11	198±18	N.S.[1]	155±12	162±12	N.S.[1]
Glucose in body fluids	37±2	54±2	17	15±1	47±1	32	25±5	51±2	26
Total increase in glucose	—	—	89	—	—	110	—	—	49
Urine nitrogen (12 hours)	54±4	81±4	27	37±4	64±7	27	66±3	99±1	33
Extra Glucose									
Extra Nitrogen	—	—	3.3	—	—	4.1	—	—	1.5

[1] Not significant.

with a level of 79 mg. in the group injected with cortical extract. This represents an increase of 72 mg. of glycogen. Similarly, the glucose in the body fluids was found to rise from 37 mg. per 100 gm. of rat to 54 mg., representing an increase of 17 mg. of glucose. Inasmuch as it has already been shown that the muscle glycogen undergoes no significant change after treatment with cortical extract, it has not entered into the computations. The average total extra carbohydrate formed is, therefore 89 mg. per 100 gm. of rat.

Two groups of adrenalectomized rats were used in an experiment similar to that carried out with normal animals. Both groups were fasted 24 hours and the urine collected for the following 12 hours. During this time they received injections of 5 cc. per 100 gm. of body weight of a solution containing 1.4% NaCl and 0.6% $NaHCO_3$. During the succeeding 12 hours, one group received 1 cc. of cortical extract intraperitoneally every hour. The other group received injections of physiological saline.

The data have been treated in a manner similar to those of the normal rats (table 4). It will be noted that there is a lower nitrogen excretion in the untreated adrenalectomized rats than was found in the normal animals. This confirms recent experiments by Evans (40) and by Harrison and Long (41).

In these animals, as in the normal ones, the injections of cortical extract produced a decided augmentation in the nitrogen excretion. The total in-

crease in nitrogen excretion after the injection of cortical extract is very nearly the same as in the normal animals described above.

The ratio between the extra carbohydrate formed and the extra nitrogen excreted under the influence of cortical extract is 3:3 in the case of the normal rats and 4:1 in the adrenalectomized. G:N ratios of this order of magnitude would indicate that 53% and 66% of the extra protein catabolized was converted into glucose. This degree of conversion is of the same order as that found in phloridzinized dogs and is not beyond the amount of glucose that theoretically can be obtained from proteins. It is, however, at the maximum level of such a conversion and consequently it must be assumed that practically none of this extra carbohydrate was oxidized during the experimental period. That this is the case is furnished by the fact that the R.Q. of the treated animals did not increase.

The results of similar experiments on fasted hypophysectomized rats are also listed in table 4 but a discussion of these will be deferred. It might be pointed out, however, that the low G:N ratio in these animals indicates that a large proportion of the extra carbohydrate was oxidized.

Effect of cortical extract on the disposition of carbohydrate. The previous experiments have indicated that in the fasted rat the cortical hormone brings about an accumulation of glycogen in the liver while at the same time it intensifies the breakdown of protein. Since in the fasted rat the carbohydrate oxidation is at a minimal level, it is not possible in this type of experiment to determine whether or not it was affected by the cortical hormone.

The experimental approach that is capable of yielding evidence on this point has been worked out by Cori and Cori (44). These authors developed what might be termed a 'balance sheet' for the fate of a given amount of carbohydrate fed to a rat. Essentially, the method consists of feeding a known amount of glucose by stomach tube to a fasted animal. Enough glucose is given to permit absorption over a 4-hour period with sufficient excess so as to leave about 100 to 200 mg. unabsorbed at the end of the experiment. During this period the respiratory exchange is determined and, at the end of the experiment, the animal is killed and the glycogen content of the whole liver and of a gastrocnemius muscle as well as the blood glucose and the unabsorbed glucose in the gastrointestinal tract are determined.

From these data it is possible, if certain assumptions are made, to calculate the disposition of the glucose absorbed. These assumptions are, *a*) that the muscles represent 50% of the body weight, and *b*) that glucose is distributed in an amount equal to that found in the blood in 50% of the body weight. When calculations are made on this basis it is possible, after subtraction of the carbohydrate levels found in fasted animals, to determine: *a*) the proportion of glucose oxidized (from the non-protein respiratory quotient); *b*), the proportion deposited as liver glycogen; *c*), the proportion deposited as muscle glycogen; *d*), quantity of extra glucose in body fluids.

The nitrogen excretion decreases slightly as a result of glucose feeding, but the main result is a transference of a large part of the metabolism from fat to carbohydrate. Using this technic the effects of insulin (44) and APE (45, 46) on the disposition of carbohydrate have been studied.

In the experiments reported here this technic has been used to determine the influence of cortical extract on the disposition of glucose. At the time of glucose feeding the rats were injected intraperitoneally with 2 cc. of cortical extract (Upjohn) from which the alcohol had been removed by vacuum distillation at a low temperature. An hour later a further 3 cc. was injected and the animals placed in a Haldane open circuit respiration apparatus. At the end of the 4-hour period the animals were anesthetized and the tissues and blood taken for analysis and the residual glucose in the gastrointestinal tract determined. Similar experiments were performed on a group of glucose-fed rats that received saline injections, while the basal carbohydrate levels were determined on a group of rats fasted for 24 hours.

The essential data are summarized in table 5. In each case it was found that the injection of cortical extract depressed the respiratory quotient of the

TABLE 5. EFFECT OF CORTICAL EXTRACT ON THE DISPOSITION OF GLUCOSE IN THE RAT.
All values mg./100 gm. body weight/4 hours.

	Fasted (12)	Glucose-fed (8)	Glucose and cortical extract (10)
Glucose absorbed	—	756±15	684±19
Muscle glycogen	253±15	393±12	412±16
Extra muscle glycogen	—	140	159
Liver glycogen	7±1	135±5	178±7
Extra liver glycogen	—	128	171
Glucose in body fluids	37±2	57±2	72±5
Extra glucose	—	20	35
Urine nitrogen	14±0.6	13.6±0.6	13.6±0.6
CO_2 (cc.)	—	548±12	488±16
O_2 (cc.)	—	635±16	628±12
R.Q.	—	0.851±0.005	0.781±0.005
Non-protein R. Q.	—	0.866±0.005	0.766±0.005
Glucose oxidized	—	361±27	154±13
Total glucose recovered	—	649±16	519±30
% Recovery	—	86±1.8	76±2.7

glucose-fed rats. The respiratory quotient of the uninjected fed rats was at an average level of 0.855 (non-protein R.Q. 0.866). These values are in good agreement with those found by Cori and Cori (44). The animals that had been treated with cortical extract had an average respiratory quotient of 0.781 (non-protein R.Q. 0.766). This indicates that the treated animals still oxidized some glucose but this took place to a lesser extent than in the untreated rats. There was no change in the oxygen consumption indicating that the total calories consumed remained constant, but that a shift occurred in the source of energy from carbohydrate to fat. By calculation from the Zunz and Schumberg tables, it is found that the control animals oxidized 48.3% of the absorbed glucose contrasted with 25.7% in the treated animals.

The analysis of the blood and tissues bears out the conclusion that less carbohydrate was oxidized by the treated rats and that more was retained as glycogen or glucose. In order to put all experiments on a comparable basis these values are expressed in terms of 100 gm. of body weight and the distribution of carbohydrate in the liver, muscles and body fluids is expressed as

a percentage of the amount absorbed. Such a method of calculation makes allowance for differences in the quantity of glucose absorbed which in the treated rats was significantly less than in the untreated.

It will be seen (table 6) that coincident with the decrease in the proportion of carbohydrate oxidized, there is a significant increase in the proportion of absorbed glucose stored as liver glycogen. In the untreated rats this was 17% while in the treated group it was 25%.

Under the influence of the cortical hormone there was a decided rise in the amount of glucose present in the blood and tissue fluids. In the untreated rats the blood glucose at the end of the period averaged 114 mg.%, but in those injected with cortical extract the average value was 154 mg.%, and in some rats was nearly 200 mg.%. Although the absolute amount of glucose present in the blood and tissue fluids is not great, the relative change between the two groups is highly significant.

TABLE 6. PERCENTAGE DISTRIBUTION OF ABSORBED GLUCOSE IN RATS WITH *a*), NO TREATMENT, *b*), CORTICAL EXTRACT, *c*), APE

	As liver glycogen	As muscle glycogen	As glucose in body fluids	Glucose oxidized	Glucose recovered
Glucose (8)	17±1	18±1	3±0.2	48±2	86±2
Glucose+C.E. (10)	25±0.7	23±2	6±0.7	23±2	73±3
Glucose+APE[1] (5)	16±3	32±2	4±0.9	18±3	69±8

[1] From data of Russell (unpublished).

The total amount of carbohydrates in the tissues of the treated animals is greater than in the untreated, but relatively little of this extra carbohydrate was found in the muscles. Table 5 showed an increase of 19 mg. of muscle glycogen per 100 gm. of body weight, but this increase is of doubtful statistical significance. This again demonstrates that in its effect on carbohydrate metabolism, the principal change brought about by the cortical hormone is in the liver glycogen and blood glucose.

This effect of cortical extract in apparently depressing the oxidation of glucose in rats is superficially similar to that observed after the injection of anterior pituitary extract (45, 46).

For comparison of the two effects, some of Russell's data (unpublished) has been tabulated along with the present experiments (table 6). It will be noted that APE depressed the R.Q. to an even greater extent, but that in this case the extra carbohydrate was largely deposited not as liver, but as muscle glycogen.

III. THE ADRENAL CORTEX AND PANCREATIC DIABETES IN THE RAT

Hartman and Brownell (47) and Long and Lukens (48) have previously shown that adrenalectomy attenuates the severe diabetes that follows total pancreatectomy in the cat. Similar results are reported by Himwich, Fazekas and Martin (49) to follow the ligation of the adrenal veins in cats. Turcatti (50) and also Long, Lukens and Dohan (51) observed the same phenomenon in dogs, while in the toad Houssay and Biasotti (52) have reported that adrenalectomized and depancreatized animals do not

exhibit the marked hyperglycemia observed when the pancreas alone is removed. Earlier experiments upon adrenalectomized depancreatized animals are reviewed in the paper by Long and Lukens.

In the experiments of Long and Lukens the technical difficulties encountered in maintaining totally adrenalectomized cats were considerable. The administration of adequate quantities of sodium salts to cats is not easy to accomplish, and in all their experiments cortical extract in varying quantities was administered. In spite of the fact that the amounts of cortical extract were considered to be adequate for life maintenance, the attenuation of the diabetes persisted, and such attempts as were made to aggravate it by injecting increasing quantities did not succeed. Since it might reasonably be anticipated that, if the loss of the cortical tissue was the factor determining the lessened severity of the diabetes, the injection of cortical extract would enhance it, the failure of these attempts created a dilemma that was not satisfactorily resolved by the alternative suggestions put forward in this paper. In a later communication Lukens and Dohan (53) reported some success in exaggerating the diabetes of adrenalectomized and depancreatized animals. Furthermore, they found that the glycosuria of hypophysectomized depancreatized animals was also increased by injection of cortical extract, a finding that is of some significance in view of the results reported here.

In the following experiments we have made use of a preparation that is relatively new in the study of diabetes. This is the partially depancreatized rat, the technic for pancreatectomy in this species having been recently published by Shapiro and Pincus (54).

The preparation, care and use of partially depancreatized rats. The advantages to be gained by the use of a smaller and more standardized diabetic animal are obvious, and since we have performed a large number of pancreatectomies using a slight modification of the original technic, our results may be of value to others who wish to use this preparation.

a). *Effect of age, sex and strain.* The operation must be performed on young rats of from 25 to 40 days of age. This point was stressed by Shapiro and Pincus, and in our experience a comparable operation in adult animals does not produce glycosuria. While male rats have been used almost exclusively, similar results may be obtained in females, although we have the impression that severe glycosuria is more easily evoked in males.

The operation has been successfully performed on several strains of rats, although the 'Yale' strain has been chiefly used. In connection with the use of this strain, it is interesting to note that Cole and Harned (55) have concluded that they possess a 'diabetic' trait as manifested by their abnormally low glucose tolerance. While this may account for the comparative ease with which severe glycosuria may be obtained in these rats, it would hardly seem necessary to point out that the high rate of reproduction and growth in this strain indicate that they are in no way to be regarded as atypical.

b). *Operative technic.* The rats are anesthetized in an ether chamber, and placed upon their backs on a simple rat board. Etherization is continued by the open mask drop method. The abdomen is cleaned with 70% alcohol and incised in the midline for about an inch downward from the xyphoid process with fine scissors. The omentum and stomach are then gently drawn out of the abdomen and allowed to lie on the abdominal wall at the upper angle of the incision. The spleen and tail of the pancreas are grasped with the fingers and drawn out into view. With blunt dissection, using

fine mouse-toothed forceps and occasionally wiping wth dry gauze, the pancreas is freed from the spleen and its arteries and veins without injury to them. This part of the pancreas is then avulsed and the spleen is replaced while the colon is freed from the duodenum and the latter is brought out for inspection.

The blood vessels which spread fanwise in the duodenal mesentery are covered on either side with a delicate layer of pancreatic tissue, and the common bile duct crosses the duodenal vessels for a distance of about 1 to 2 cm. beneath the first portion of the duodenum. These structures must not be injured while part of this pancreatic tissue (approximately ⅓) is removed piecemeal. A separate segment of pancreas in the neighborhood of the pylorus and the right margin of the greater omentum is then avulsed. If this causes bleeding it is easily controlled with gentle pressure. The remainder of the body of the pancreas overlying the mesenteric vessels beneath the colon is then removed. Care should be taken in this area to avoid avulsion of the lymph nodes which tend to bleed.

The closure is carried out when all of the pancreas is removed excepting that portion of the head which is attached to the duodenum and its vessels. The spleen and then the stomach and duodenum are replaced in the abdominal cavity, and the aponeurosis and skin are closed with interrupted sutures of 'A' silk passed on a milliner's needle.

Several other points of technic deserve brief discussion. Neither antisepsis nor asepsis is necessary, for the procedure is well tolerated by rats if simple 'soap-and-water' cleanliness is observed. It is best not to remove the spleen during the operation because this often precipitates an exacerbation of Bartonella infection which is present in latent form in many rats. It is advisable to take considerable pains to remove all of the body and tail of the pancreas, in order that more pancreas may be removed in the future from the accessible region of the head of the pancreas, if the animal does not become sufficiently diabetic.

This operation has been performed on 142 rats with a mortality within the first month of 15%. The chief cause of early death is either hemorrhage or injury to the blood vessels of the duodenum. Later death may ensue from injury to the bile ducts or intestinal obstruction. Most fatalities, however, occur within the first 72 hours postoperative.

A more complete, if not a total, pancreatectomy can probably be effected by the use of a suction tube (56), but since our object was to obtain animals that would survive in spite of severe glycosuria, for long periods without insulin treatment, we have not endeavored to remove all the pancreas. Furthermore the operation described by Richter and Schmidt (56) destroys the external secretion of the pancreas, an undesirable consequence if the rats are used for studies extending over a long period.

c). Postoperative care and behavior. Following the operation, the rats were fed their ordinary diet of Dog Chow which, since they still possess an adequate external pancreatic function, is readily assimilated. As may be expected, growth is retarded for a few days after operation, but most animals begin to gain weight again after this period. The rate of growth in most instances is much less than that of animals subjected to a dummy operation.

When the rats had overcome the effects of the operation, they were placed in individual metabolism cages so arranged as to allow separate collection of urine. The 24-hour specimens of urine were collected in flasks containing an adequate quantity of toluene, the collecting funnels being washed down with 5 to 10 cc. of water at the end of each period and the washings added to the specimens.

Since it was not possible to obtain uncontaminated specimens of urine when the rats were fed either Dog Chow or a powdered diet, all the diets used were in paste

form and of demonstrated ability to promote normal growth and health in intact rats. The most commonly used diet in these studies consisted of 100 gm. of cornmeal, 200 gm. of lean minced beef, 100 gm. of casein and 4 gm. of cod liver oil. To this was added 2 gm. of Osborne-Mendel salt mixture and 250 gm. of water, the whole being mixed into a paste. This diet contains 11% of carbohydrate, 24% of protein and approximately 2% of fat. The total available carbohydrate is 25%. Other diets containing different proportions of the main foodstuffs were constructed and used in a similar manner. The quantity of food ingested daily was measured, while at the same time the urine specimens were separated and the rats weighed. When blood was required, it was obtained by snipping the tail and collecting approximately 0.2 cc. in a tube coated with potassium oxalate.

d). *Development of glycosuria.* Not all rats subjected to the operation described aboxe develop spontaneous glycosuria, and in a series of 117 rats studied only 45% excreted glucose over periods long enough for them to be classed as permanently gly-

TABLE 7. DAILY VARIATION OF GLYCOSURIA IN PARTIALLY DEPANCREATIZED RATS

Rat No.	Age,[1] days	Body weight,[1] gm.	Period, days	Urine volume, cc./day	Urine Glucose		Available glucose excreted, %	Changes in body weight, gm./day	Diet, gm./day
					gm./day	Coef. varia. %			
76	162	271	63	92	4.3±0.1	15.8	37.2	+0.1	45
78	401	280	31	94	5.1±0.3	27.4	31.4	+1.9	50
79	170	324	50	83	4.4±0.1	14.7	37.9	−0.9	45
81	224	329	49	86	4.4±0.1	15.6	40.7	−0.8	42
85	230	321	40	77	2.2±0.1	30.0	17.0	+1.7	50
102	61	220	39	68	4.0±0.1	13.6	36.3	+1.6	35
100	73	230	21	99	6.6±0.3	17.7	50.7	+0.6	40
103	82	225	30	82	5.4±0.2	22.4	41.5	+1.4	40
111	96	182	25	99	6.9±0.1	8.1	50.7	+0.2	42

[1] Age and body weight at beginning of period.

cosuric. On the other hand, those animals that do not exhibit glycosuria under ordinary circumstances are of value for certain types of experiments, inasmuch as their reduced pancreatic function renders them particularly susceptible to the action of any 'diabetogenic' agent. Examples of the use of such animals will be found in this paper. It may be added that it has been our invariable custom to inject all rats not exhibiting glycosuria with a crude saline extract of the anterior pituitary. Since normal rats never in our experience exhibit glycosuria following such injections and, since the majority of aglycosuric, partially depancreatized rats do, this procedure forms a convenient method of eliminating those animals in which the amount of pancreas removed has been inadequate. Thus, in our series of 117 animals, 53 exhibited permanent glycosuria, 51 developed glycosuria after APE injection (some of these becoming permanently glycosuric after this treatment), while 14 did not respond to the injection of APE.

Since the validity of many deductions from experiments upon permanently glycosuric animals is dependent, not only on the permanence of glycosuria under ordinary circumstances but also on the relative constancy of this glycosuria from day to day, it is necessary to establish these points before presenting these conclusions.

Table 7 lists a series of observations on partially depancreatized rats with varying degrees of glycosuria. It will first be noted that the glycosuria may reach an extremely high degree, 5 or 6 gm. a day being not uncommon, while occasionally a rat may excrete 10 gm. or more of glucose a day. The most revealing fact as to the severity of

the diabetes is afforded by calculating the percentage of the available glucose of the diet that is excreted and this may, in the most extreme cases, be more than 50% of the amount ingested. Under these circumstances it is not surprising to find a large urine volume, a voracious appetite and a marked retardation of growth. All the latter are well-known associates of the severely diabetic state in dogs and cats, but there are certain differences in the course of events in the diabetic rat. The length of life and general state of well-being of even the severely diabetic rats do not appear to be markedly affected, indeed, none of our animals have relapsed at any time into diabetic acidosis or coma. Although growth is retarded or may practically cease some of our rats have survived for long periods after pancreatectomy, one for over two years. Another fact of some interest is the complete absence of ketonuria, which does not develop to more than a normal extent on fasting.

All the above findings are probably explicable on the grounds that although a severe diabetes is present, the amount of pancreas left is sufficient to prevent the onset of acidosis and coma. The islet tissue of the pancreatic remnants displayed the well-known pathological changes previously described in partially depancreatized dogs. There may be found hypertrophy and all degrees of islet cell degeneration from the most minor to complete destruction and hyalinization.

Once established, the daily excretion of glucose may be held within fairly close limits by careful technic and, indeed, within quite as close limits as that of depancreatized dogs maintained on insulin. This fact renders these small animals of particular value in the study of certain problems not only on account of the large amount of information that we already possess concerning their metabolism, but also by reason of the opportunity they afford for the use of a pure strain of experimental animals. A further consideration is the large number that may be observed at one time as well as the economy of their maintenance.

With very few exceptions glycosuria is maintained only so long as the animals are fed. When fasted, the glycosuria has usually disappeared within 12 hours, and in all cases within 24 hours. Nevertheless, a study of the blood glucose levels during fasting reveals the abnormal state of the metabolism. Thus, in a series of 6 partially depancreatized rats, the average blood glucose at the end of a 24-hour fast was 170 mg.% (range 144–250 mg.%). None of these rats excreted any glucose during this period. The blood glucose level in a similarly treated group of normal rats was 73 mg.% (range 55–92 mg.%). Since the renal threshold for glucose in the rat appears to be placed at a higher level than in some mammals (57), it is not surprising that glycosuria disappears. In the fed partially depancreatized rat very high blood glucose levels were observed, often exceeding 500 mg.%.

Effect of adrenalectomy on the glycosuria of partially depancreatized rats. We have already pointed out the value of the partially depancreatized rat in studies of this kind, and it is also well to recall that following adrenalectomy the rat can be maintained in the majority of instances by the incorporation of sodium salts into the drinking water. This combination of circumstances appeared to be adapted to the further study of two important questions. *a*). Does an attenuation of pancreatic diabetes occur in an animal deprived of cortical tissue, but maintained in good health by the administration of sodium salts? *b*). If an attentuation is observed, can the diabetes be reestablished by the administration of cortical extract? A third problem that will be considered later is the effect of the so-called 'diabetogenic' action of anterior pituitary extracts in these animals.

TABLE 8. EFFECT OF ADRENALECTOMY WITH SUBSEQUENT MAINTENANCE BY 0.9% NaCl ON THE GLYCOSURIA OF PARTIALLY DEPANCREATIZED RATS

Rat no. initial wt. and diet	Condition	Length of period, days	Change in body wt., gm./day	Urine glucose gm./day	Food intake, gm./day	Available glucose excreted, %
38 238 gm. 11% COH	Intact.	6	−1.4	2.8±0.2	34±2.0	32
	Adrenx.	10	+3.2	0.3±0.1	38±1.0	3
76 258 gm. 11% COH	Intact	50	+0.3	4.1±0.1	44±1.0	37
	Adrenx.	7	+1.9	1.1±0.3	42±1.0	10
97 27 gm. 78% COH	Intact	9	−1.9	5.6±0.3	18±1.0	35
	Adrenx.	9	+2.2	3.4±0.2	19±0.5	20
111 186 gm. 53% protein	Intact	19	+1.6	7.4±0.2	16±0.5	75
	Adrenx.	5	+1.2	2.9±0.3	12±0.0	39
123 176 gm. 25% COH	Intact	23	+0.4	6.4±0.1	35±0.0	57
	Adrenx.	10	+3.1	3.5±0.2	33±0.6	33

The partially depancreatized rats were prepared in the manner already described, and their daily output of glucose on a constant regime was observed prior to adrenalectomy. Following the removal of both adrenal glands (at one operation), the animals were restored to the same regime, only this time 0.9% NaCl solution was substituted for the tap water previously used as drinking fluid. The effect of this procedure on the glycosuria was then observed (table 8).

TABLE 9. EFFECT OF ADRENALECTOMY WITH SUBSEQUENT MAINTENANCE BY CORTICAL HORMONE OR 0.9% NaCl ON THE GLYCOSURIA OF PARTIALLY DEPANCREATIZED RATS

Rat no. initial wt. and diet	Condition	Length of period, days	Change in body wt., gm./day	Cortical hormone, cc. or mg./day	Urine glucose, gm./day	Food intake, gm./day	Available glucose excreted, %
8 340 gm. 11% COH	Intact	20	+ 0.8	0	7.4±0.4	52±0.8	57
	Adrenx.	6	− 0.5	5 cc. C.E.	4.5±0.7	51±2.0	36
	Adrenx.	11	+ 3.1	0	0.7±0.2	47±0.8	6
	Adrenx.	13	− 0.2	5 cc. C.E.	3.2±0.2	44±0.0	29
	Adrenx.	13	+ 1.1	0	1.2±0.2	43±0.4	11
	Adrenx.	3	−14.0	3.3 mg. corticosterone	4.7±1.0	39±2.1	48
	Adrenx.	15	+ 3.0	0	1.4±0.2	44±0.0	13
50 310 gm. 11% COH	Intact	15	− 1.3	0	4.7±0.4	50±1.1	38
	Adrenx.	6	− 1.3	5 cc. C.E.	4.9±0.3	46±1.7	43
	Adrenx.	10	+ 1.2	0	1.8±0.2	41±2.0	18
77 304 gm. 11% COH	Intact	19	+ 0.7	0	4.6±0.2	54±0.0	34
	Adrenx.	4	− 4.5	3.3 mg. corticosterone	3.8±0.6	46±3.1	33
	Adrenx.	5	+ 4.6	0	0.4±0.4	38±3.5	4
90A 440 gm. 25% COH	Intact	6	− 1.9	0	2.6±0.1	42±0.0	19
	Adrenx.	7	+ 0.9	5 cc. C.E.	1.1±0.2	40±0.0	9
	Adrenx.	4	− 1.5	10 cc. C.E.	2.4±0.4	39±0.0	19

In another series of experiments (table 9) the animals were given either cortical extract, corticosterone or dehydrocorticosterone in their drinking water immediately after adrenalectomy. Subsequently this was withdrawn and 0.9% NaCl given in its place.

Finally, in another small series very large quantities of cortical extract were administered in an attempt to drive the glycosuria to higher levels than those existing prior to adrenalectomy. Such a demonstration, it was felt, would greatly strengthen the view that the effect of the cortical extract on glycosuria was not limited to any effect its administration might have on

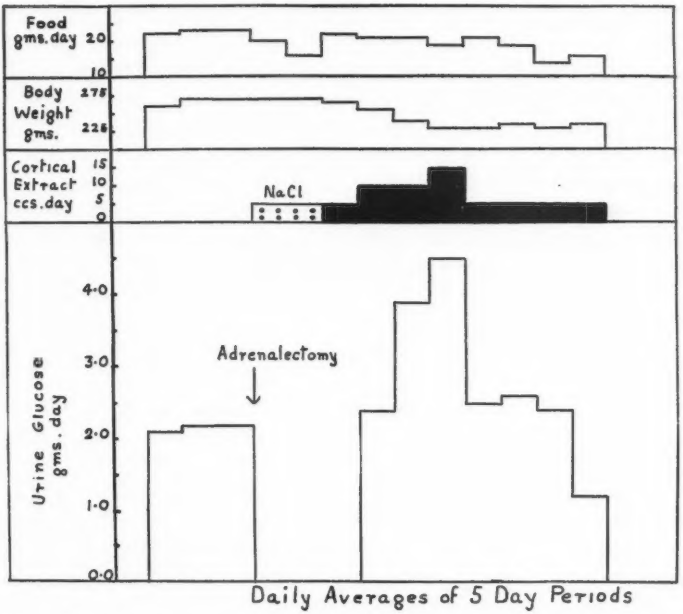

Fig. 1. Effect of adrenalectomy and subsequent treatment with sodium chloride or cortical hormone on the glycosuria of a partially depancreatized rat.

restoring an adrenalectomized animal to normal, but was directly related to its influence on carbohydrate metabolism. An illustrative example of the effect of increasing quantities of cortical extract on the level of glycosuria is seen in figure 1.

The results (tables 8 and 9) show that following adrenalectomy, when the animals are maintained on sodium chloride, there is an unmistakable reduction in glycosuria even when allowance is made for any alteration in the food ingested. The variation in food intake has been compensated by expressing the glycosuria as a percentage of the available glucose ingested. As a matter of fact, the average glycosuria during the period after adrenalectomy conceals to some extent the marked alteration in the glycosuria that ultimately occurs. This rapidly declines during the first day or two, and subsequent to

this in many animals no glycosuria is present in spite of the fact that the food ingested remains at approximately the same level (fig. 1.)

In those experiments (table 9) in which cortical extract was administered by mouth immediately after adrenalectomy the degree of glycosuria that continues is largely determined by the amount of extract given. Furthermore, the quantity of extract required to maintain the glycosuria at pre-operative level, although varying somewhat from animal to animal, is considerably greater than would be anticipated. Thus, in *rat* 90A, 10 cc. a day by mouth were necessary to restore the preoperative level. The withdrawal of the extract and substitution of sodium chloride is followed by a marked reduction in glycosuria which is again augmented when the hormone is readministered.

It is estimated from a considerable number of experiments that, when administered by mouth to the rat, approximately 3 cc. per 100 gm. are required to restore the pre-operative level of glycosuria, and hence it may be assumed that quantities of this order are necessary to supply hormone at a rate comparable to that furnished by the animal's own adrenals.

It is a point of considerable interest that the glycosuria may also be maintained or reaugmented by the administration of certain crystalline substances isolated from the adrenal cortex. Through the kindness of Dr. E. C. Kendall, we have had an opportunity to test corticosterone and dehydrocorticosterone in this manner. Both these compounds in quantities of approximately 3 mg. a day by mouth are sufficient to maintain or restore the glycosuria to its pre-operative level.

The demonstration that crystalline substances which are known to maintain life and a normal electrolyte balance in adrenalectomized animals are also capable of preserving the glycosuria of adrenalectomized-depancreatized animals affords an explanation of the observations of Long and Lukens (48) that the administration of presumably adequate quantities of cortical extract did not exacerbate the glycosuria of totally adrenalectomized-depancreatized cats. It is apparent that the quantity of hormone they administered, although lengthening the life of the animals, was inadequate to completely restore the glycosuria. The above results therefore remove the necessity for their suggestion that the adrenal cortex secretes separate factors for the control of the electrolyte and carbohydrate metabolism.

The ability of increasing quantities of cortical extract to increase the glycosuria to amounts greater than those existing prior to adrenalectomy (fig. 1) furnishes an indication that its effect upon carbohydrate metabolism is not simply due to any general improvement in the condition of the animal. Indeed, in these experiments where the rats maintained on sodium chloride ate well and to all outward appearances were in good health, it does not seem possible that the subsequent administration of the hormone affected carbohydrate metabolism merely by improving their well-being. However, it is not entirely possible to avoid this criticism so long as adrenalectomized animals are employed and consequently, it is of importance to show that alterations in metabolism can be produced by cortical extract in depancreatized animals with intact adrenals.

Effect of cortical extract on partially depancreatized rats. Two types of experiments were performed. In the first group fed animals were used and the effect on glycosuria of the addition of cortical extract to the drinking water was determined. In the second group the animals were fasted and injected with multiple doses of cortical extract and the effect of this procedure on the blood glucose and urinary nitrogen followed.

a). Fed rats. Partially depancreatized rats that had exhibited a constant degree of glycosuria under a given regime for a sufficiently long period were given either cortical extract (Upjohn) or the crystalline compound corti-

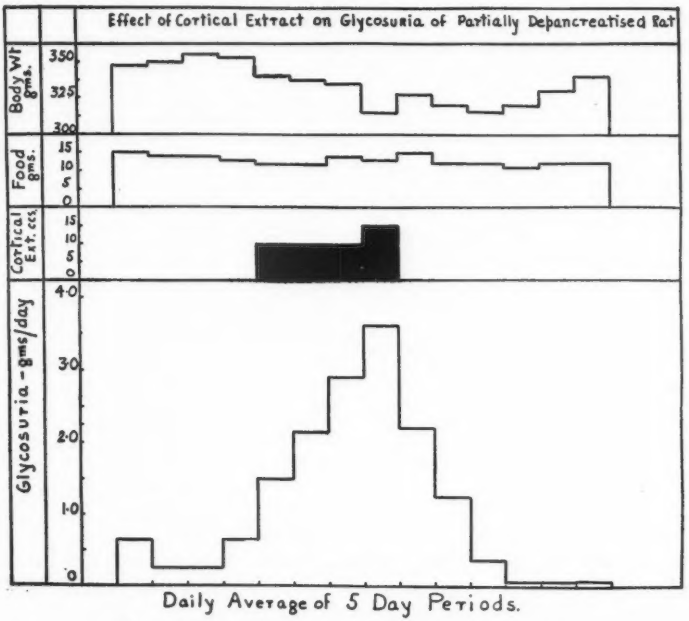

Fig. 2. Effect of cortical extract on the glycosuria of a partially depancreatized rat.

costerone by placing them in the drinking water in varying amounts. After a sufficient period on this regime the hormone was withdrawn. The results of these experiments are given in table 10 and figure 2. There would seem to be no doubt that the administration of sufficiently large quantities of cortical extract or corticosterone brings about a marked increase in glycosuria. The increase in glycosuria following this treatment is in most instances accompanied by a decline in the weight of the animal which appears to be out of proportion to the increased glucose excretion and which is regained on withdrawal of the hormone. Similar losses in the weight of normal rats treated with large quantities of cortical extract by mouth have been reported by Ingle (58). It will also be noted that the ingestion of a solution of epinephrin of the concentration present in the cortical extract (1:1 million) did not affect the glycosuria.

Since the food intake remained approximately constant throughout the various experimental periods, and since in two animals determinations of the nitrogen excretion did not reveal any increase in protein catabolism, it may be assumed that the increased glycosuria was a result of a further depression of the already deficient ability of the animals to utilize carbohydrate. Such an assumption would be in keeping with the results already described on the effect of cortical extract in glucose-fed animals (see part II).

Ingle, Higgins and Kendall (59) have shown that in normal rats the administration of large quantities of cortical extract brings about an involution

TABLE 10. EFFECT OF CORTICAL EXTRACT AND CORTICOSTERONE ON THE GLYCOSURIA OF THE FED PARTIALLY DEPANCREATIZED RAT

Rat no., initial wt. and diet	Length of period, days	Change in body wt., gm./day	Cortical hormone, cc. or mg./day	Urine glucose, gm./day	Food intake, gm./day	Available glucose excreted, %	Urine nitrogen, gm./day
32	18	+0.9	0	1.2±0.1	59±0.4	8	—
454 gm.	9	+0.1	5 cc. C.E.	2.9±0.1	60±0.1	19	—
11% COH	9	−2.0	10 cc. C.E.	3.2±0.1	59±0.1	22	—
	15	+1.3	0	1.8±0.1	60±0.0	12	—
	5	+0.6	3.3 mg. corticosterone	2.8±0.3	61±0.0	18	—
	50	+0.4	0	1.9±0.1	59±0.5	13	—
	6	−1.7	10 cc. C.E.	3.9±0.3	60±0.0	26	—
	20	+1.3	0	1.8±0.2	60±0.2	12	—
	5	+1.2	50 cc. 1:1 million epinephrin	1.3±0.1	59±0.0	9	—
112	15	+1.3	0	3.6±0.1	13±0.4	45	0.85
300 gm.	10	−3.4	10 cc. C.E.	4.4±0.2	12±0.0	65	0.87
53%	5	−1.0	15 cc. C.E.	5.0±0.2	12±0.0	67	—
protein	5	−1.8	20 cc. C.E.	4.8±0.2	12±0.0	64	—
	20	+1.2	0	0.9±0.1	12±0.0	12	—
	15	−1.9	10 cc. C.E.	2.2±0.3	12±0.0	29	—
	5	−0.8	15 cc. C.E.	3.3±0.2	12±0.3	44	—
	5	−2.0	20 cc. C.E.	3.9±0.1	11±0.3	58	—
	15	+1.5	0	0.2±0.1	12±0.0	3	—
118	13	+4.2	0	6.1±0.2	21±0.0	47	1.52
236 gm.	3	+0.3	3.3 mg. corticosteron	7.7±0.1	21±0.0	59	1.52
53% protein	5	±0.0	10 cc. C.E.	10.0±1.0	19±0.2	85	1.45
	5	±2.2	0	6.4±0.1	18±0.3	57	1.28

of the adrenal cortices to a degree comparable to that observed after hypophysectomy. This phenomenon may in part explain the apparently large quantities of exogenous cortical hormone that are required to significantly enhance the glycosuria of partially depancreatized rats inasmuch as quantities greater than the daily output of their own adrenal cortices are required in order to produce an effective excess of the hormone in the body fluids.

b). *Fasted rats.* The effect of cortical extract on the blood glucose and nitrogen excretion of partially depancreatized rats was determined as follows.

Six partially depancreatized rats were fasted for 36 hours. Between the 24th and 36th hour subcutaneous injections of either 0.5 cc. per 100 gm. of physiological saline or cortical extract were given hourly. The animals were first injected with saline, but at a later date they were again fasted and the

cortical extract injected. Thus the response both to saline and cortical extract was determined in the same animal.

The urine was collected for nitrogen determinations in two 12-hour periods, the first extending between the 12th and 24th hours of fasting, and the second during the injection period (24–36 hours). Blood was obtained for glucose analysis by snipping the tail at the beginning and end of injections.

The nitrogen excretion of the saline controls (table 11), unlike that of normal fasted rats, did not always decline during the second period of collection but either remained constant or increased slightly. The blood glucose at the end of a 24-hour fast was much higher than that of normal rats but usually fell during the subsequent period of saline injection. Although all the rats used had glycosuria when fed this disappeared after fasting for 24 hours.

TABLE 11. EFFECT OF CORTICAL EXTRACT ON THE BLOOD GLUCOSE AND NITROGEN EXCRETION OF FASTED DEPANCREATIZED RATS

Rat No.	SALINE				CORTICAL EXTRACT			
	Urine nitrogen, mg./100 gm./12 hours		Blood glucose, mg./100 cc.		Urine nitrogen, mg./100 gm./12 hours		Blood glucose, mg./100 cc.	
	Fore-period	Injection period	Fore-period	Injection period	Fore-period	Injection period	Fore-period	Injection period
118	50	49	—	—	52	84	362	307[1]
121	42	48	250	293	58	68	199	242[1]
122	52	45	175	122	42	68	153	275
125	49	54	144	190	31	70	91	227
128	77	87	148	80	78	80	—	—
129	35	47	149	84	54	82	149	282[1]

[1] Glycosuria during injection period.

In the cortin-treated experiments a marked increase in nitrogen excretion occurred in all but one instance during the injection period. The blood glucose rose to high levels and in two instances slight glycosuria occurred, a phenomenon that has not been observed in fasting, partially depancreatized rats injected with anterior pituitary extract.

The one rat, *128*, that did not show an increased urinary nitrogen excretion with cortical extract was, prior to injection, already excreting nitrogen at a rate as great as that found in the other rats after treatment.

The effect of various steroids on partially depancreatized rats. A large number of crystalline steroids have been isolated from the adrenal cortex (60). We have, through the kindness of Dr. E. C. Kendall and Dr. O. Wintersteiner, had the opportunity to test the activity of certain of these compounds in promoting glycosuria both in depancreatized rats with intact adrenals and adrenalectomized-depancreatized rats. The latter were maintained with NaCl prior to treatment. The structural formulae of these compounds are shown in figure 3 along with that of progesterone—a substance that was also tested. The compounds that were sufficiently soluble were added to the drinking fluid which was either water or 1% NaCl. Otherwise they were

suspended in oil and injected subcutaneously. They were administered to the rats for 3 to 5 days after a sufficiently long foreperiod (5–10 days) on a constant dietary regime to establish the basal level of glycosuria. Following treatment, the glycosuria was observed for another 5 to 10 days. The figures given are the daily average of these periods.

The results of these experiments are to be found in table 12. It will be observed that corticosterone, dehydrocorticosterone, and compound *F* of Wintersteiner and Pfiffner all exacerbate the glycosuria of the two types of preparations used. In this test progesterone and the fully saturated compound *A* of Wintersteiner and Pfiffner are inactive. Desoxycorticosterone, a substance that is as potent in maintaining the life of adrenalectomized animals as

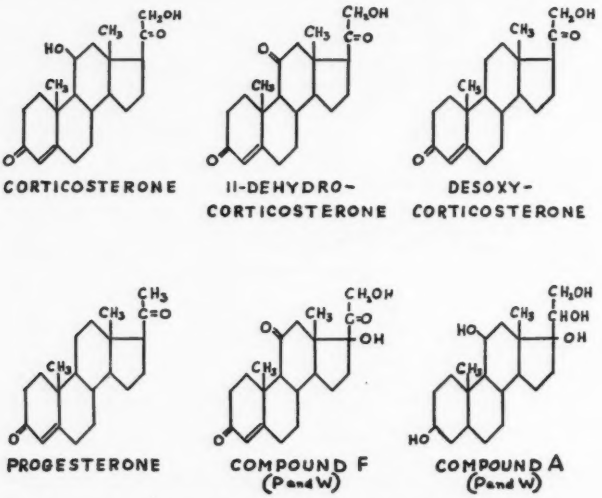

Fig. 3. Structural formulae of certain steroids isolated from the adrenal cortex.

corticosterone, dehydrocorticosterone, or compound *F*, gave a positive test only once out of 7 trials on 5 rats. While it cannot be denied that the relative insolubility of this compound may account for the large number of negative results, sufficient is absorbed when administered either in oil or as a pellet to maintain the life of adrenalectomized dogs (61). This may indicate that the effective concentration of this substance required to influence carbohydrate metabolism is greater than that necessary to support adrenalectomized animals.

Progesterone in relatively large amounts has also been shown to support the life of adrenalectomized rats (62, 63), but in the quantities given in these experiments it had no effect on glycosuria. Recently, however, Gaunt, Remington and Edelman (64) have found that the injection of large quantities (50 mg.) into normal ferrets increased the liver glycogen. No effect, however, was found in rats.

TABLE 12. EFFECT OF CERTAIN ADRENAL STEROIDS ON THE GLYCOSURIA OF *a*), PARTIALLY DEPANCREATIZED RATS, *b*), ADRENALECTOMIZED-DEPANCREATIZED RATS

Steroid injected	Amount, mg./day	Rat No.	Glycosuria, gm./day			Mode of administration
			Before injection	During injection	After injection	
A. Partially Depancreatized Rats						
Corticosterone[1]	3.3	32	1.8±0.1	2.8±0.3	1.9±0.1	Per os.
	3.0	113	0.0	0.6±0.2	0.0	In oil
	3.3	118	6.5±0.1	7.7±0.1	7.4±0.2	Per os.
Dehydro-corticosterone[1]	0.5	87	0.8±0.1	2.8±0.2	2.5±0.2	Per os.
Desoxy-corticosterone	3.0	112	0.0	0.3±0.1	0.1±0.1	In oil
	3.0	112	0.0	0.0	0.0	In oil
	3.0	113	0.0	0.0	0.0	In oil
	3.0	113	0.0	0.0	0.0	In oil
	3.0	118	6.4±0.1	7.7±0.2	7.7±0.2	In oil
	3.0	121	5.0±0.2	4.4±0.1	5.3±0.1	In oil
	5.0	122	3.7±0.1	3.8±0.3	3.7±0.4	In oil
Progesterone	3.0	112	0.0	0.0	0.0	In oil
	3.0	113	0.0	0.0	0.0	In oil
B. Adrenalectomized-depancreatized Rats						
Corticosterone	3.3	8	0.3±0.1	4.8±1.0	2.3±0.2	Per os.
	0.5	76	0.5±0.3	2.1±0.1	1.4±0.1	Per os.
	3.0	102	0.0	0.3±0.1	0.0	Per os.
Dehydro-corticosterone	0.5	76	0.9±0.3	1.5±0.3	0.4±0.2	Per os.
	3.3	77	0.4±0.4	2.1±0.3	0.2±0.1	Per os.
	0.5	77	0.0	0.9±0.2	—	Per os.
	0.5	77	0.9±0.2	3.2±0.2	2.3±0.2	Per os.
Compound *F* (P & W)[2]	2.0	76	0.4±0.2	2.6±0.3	1.2±0.1	Per os.
Compound *A* (P & W)[2]	2.0	76	0.4±0.2	0.0	—	Per os.
Progesterone	3.0	76	0.3±0.1	0.0	—	In oil
	3.0	102	5.1±0.1	2.3±0.5	0.0	In oil

[1] From Dr. E. C. Kendall. [2] From Dr. O. Wintersteiner.

It may be concluded that certain steroids of the adrenal cortex that are capable of supporting life and a normal electrolyte balance in adrenalectomized animals also affect the carbohydrate metabolism of partially depancreatized rats. There are indications, however, that not all the cortical steroids capable of maintaining adrenalectomized animals are of equal potency in their effect on carbohydrate metabolism.

IV. THE ADRENAL CORTEX—ANTERIOR PITUITARY RELATIONSHIP IN THE CONTROL OF METABOLISM

The removal of the hypophysis is followed by marked alterations in metabolism. The chief of these are a rapid reduction of the carbohydrate levels when the animals are fasted, together with an abnormally high respiratory quotient. They also exhibit extreme sensitivity to insulin, and when depancreatized the diabetes that develops is of a mild character (65).

It will be noted that some of the effects are very similar to the changes seen in adrenalectomized animals and this, together with the fact that the anterior pituitary has a tropic action on the adrenal cortex, raises the question as to how far the effects of hypophysectomy on metabolism are brought about by the ensuing hypofunction of the adrenal cortical tissue.

The problem has been approached in three ways: *a*), by a study of the effect of the cortical hormone on the metabolism of fasting hypophysectomized rats; *b*), the effect of this hormone on hypophysectomized-depancreatized rats; and *c*), the effect of adrenalectomy on the 'diabetogenic' action of anterior pituitary extract.

The effect of cortical extract on fasting hypophysectomized rats. The rats used were hypophysectomized by the para-pharyngeal approach and all had recovered from the effects of the operation before use. Untreated hypophysectomized rats withstand the effects of fasting very poorly. The rapid depletion of their carbohydrate stores soon brings on hypoglycemia, and death may result. There is also a considerable fall in body temperature as the fast proceeds. In the group of untreated animals, the body temperature had fallen to an average of 31.4°C. at the end of 24 hours and in another group to 30.3°C. after 42 hours.

TABLE 13. EFFECT OF CORTICAL EXTRACT ON THE BODY TEMPERATURE, WEIGHT LOSS AND CARBOHYDRATE LEVELS OF FASTED HYPOPHYSECTOMIZED RATS

No. of animals	Total fast, hours	Period of injection, hours	Body temp. end of expt., °C.	Weight loss, gm./100 gm.	Glycogen, mg. %		Blood glucose, mg. %	Notes
					Liver	Muscles		
8	24	—	31.4±0.6	12.0±1.7	113± 17	283±33	50± 8	Controls
5	24	12–24	—	—	808±240	374±30	94± 8	1 cc. C.E. every hour
6	24	0–24	34.8±0.01	19.5±0.3	583±124	439±12	101±12	1 cc. C.E. every hour
1	24	0–24	33.0	26.0	1520	450	107	0.03 mg. corticosterone every hour
2	36	24–36	34.5±0	25.0±4.0	1330±240	449±24	105±1	1 cc. C.E. every hour
6	42	—	30.3±1.7	8.3±1.6	87± 17	150±15	44±8	Controls
6	42	24–42	35.5±0.02	17.0±0.9	990±251	280±26	110±4	1 cc. C.E. every hour

In contrast to the poor condition of the untreated group, the rats receiving injections of 1 cc. of cortical extract every hour resembled in all outward appearances normal fasted animals, and their body temperature averaged nearly 35°C. In marked contrast to the apathetic condition of the control group they showed normal activity but lost a good deal more weight. This result might have been anticipated from the earlier work of Baird, Cloney and Albright (66) who showed that hypophysectomized rats could be protected against the lethal effects of cold by cortical extract. Ingle (67) has also shown that the work capacity of the muscles of hypophysectomized rats is greatly enhanced by the administration of cortical extract.

The effects of the injection of cortical extract on the carbohydrate levels were as striking as their influence on the general condition of the animals. These are summarized in table 13.

It will be observed that the glycogen content of the liver was maintained at a high level, and that the blood glucose, instead of being reduced to hypoglycemic levels was elevated to values well above those found in normal animals fasted for a similar period. A similar result was observed in one animal treated with crystalline corticosterone.

That this effect is not merely a retention of preformed carbohydrate is illustrated by the fact that in rats first fasted to deplete their carbohydrate stores, the subsequent injection of cortical extract elevates these stores to super-normal levels.

The effect on the muscle glycogen is not so clearly marked as on the liver glycogen and blood glucose, and while the muscle glycogen did not decline as abruptly as in the untreated animals, it was not maintained at the high level that can be induced by the injection of APE (glycostatic effect) (68).[2]

As in the previous studies on normal and adrenalectomized rats these results show that under the influence of the cortical extract a new formation of carbohydrate has occurred, and since the most likely source of this is the tissue protein, similar studies were made on the urinary nitrogen excretion of another two groups of fasted hypophysectomized rats. The only difference from the previous group of experiments was that the hypophysectomized rats were first fasted for 24 hours, the urine excreted during the last 12 hours being collected. Following this one group was injected with 1 cc. of cortical extract every hour for 12 hours, the urines of these and of the control uninjected group also being collected. At the end of the period the animals were killed and the carbohydrate levels determined.

The data show (table 4) that as in the first group of animals (table 13) there is a striking increase in the liver glycogen and blood glucose with little or no alteration in the muscle glycogen. The urine nitrogen which in the untreated animals was slightly higher than in normal fasted rats increased 50% in the group injected with cortical extract. When the data are summarized in the manner already described for normal and adrenalectomized rats (table 4), it will be seen that the ratio between the extra glucose and nitrogen is about 1.5 in contrast to the much higher ratios found in the former. Since it is unlikely that the catabolism of the tissue protein of hypophysectomized rats yields a much smaller proportion of glucogenic amino acids than that of normal animals, it may be assumed that a large proportion of this extra glucose has been oxidized. It will be recalled that others (45, 46, 73) have shown that the R.Q. of hypophysectomized rats indicates that these animals oxidize an abnormally high proportion of carbohydrate both in the fasted and fed state. Up to the present, determinations of the R.Q. of fasted hypophysectomized rats treated with cortical extract have not been made but it may be assumed that the low G:N ratios found both in these experiments and in fasted phloridzinized and hypophysectomized dogs (74) are to be attributed to this cause.

The 'diabetogenic' action of cortical extract in hypophysectomized-depancreatized rats. The 'diabetogenic' action of anterior pituitary extract is particularly marked in hypophysectomized-depancreatized animals, and since Long and Lukens (48) have suggested that this effect may in part be mediated by the adrenal cortex, it is of considerable interest to determine whether the

[2] Soon after a preliminary note of these experiments appeared (69), similar results were reported by Russell and Craig (70), and by Corey and Britton (71). Grollman (72) has reported negative resultes but the amount of hormone given was probably too small to produce measurable effects on carbohydrate metabolism.

cortical hormone can influence the mild diabetes of these preparations. Lukens and Dohan (53) have already reported that an increased glycosuria and nitrogen excretion, as well as an elevated blood glucose level, may be induced by treatment of 'Houssay' cats with cortical extract.

Our experience with fasted 'Houssay' rats is quite in accordance with these experiments. These rats were first fasted for 4 to 6 hours and the urine collected. This short period of preliminary fasting was necessary on account of the rapidity with which the blood glucose level of these rats declines. Following this, the animals were injected with 1 cc. of cortical extract every hour for 6 hours, and the urine collected during this period and for the following 2 hours. A further specimen was then collected over the following 13 hours of fasting. Blood samples for glucose determination were drawn at suitable intervals from the tail. As table 14 shows, not only did the rats develop marked hyperglycemia as a result of the injections, but this was

TABLE 14. EFFECT OF CORTICAL EXTRACT AND ANTERIOR PITUITARY EXTRACT ON BLOOD GLUCOSE AND NITROGEN EXCRETION OF FASTING HYPOPHYSECTOMIZED-DEPANCREATIZED RATS

Rat No.	Blood glucose, mg.%					Urine nitrogen, mg./100 gm./hr.			Notes
	Start of fast	Start of inject.	End of inject.	4 hr. later	11 hr. later	Fore-period	Inject. period	Post inject. period	
118	169	77	153	273	112	2.7	3.6	7.0	Cortical Extract 1 cc. every hr. between 6th and 12th hr. of fasting.
119	—	66	197	95	80	4.2	6.9	5.7	
129	—	182	278	113	—	—	5.6	—	
119	76	53	56[1]	—	—	5.1	2.6	—	1 cc. APE at 6th hour of fast.

[1] Hypoglycemic shock.

accompanied by a well-marked increase in urine nitrogen excretion. On another occasion one of the animals, *119*, was injected with a saline extract of the anterior pituitary instead of cortical extract. The behavior of the blood glucose and urine nitrogen was the exact opposite to that previously observed with cortical extract. The blood glucose fell and hypoglycemic shock occurred necessitating glucose injection, while the nitrogen excretion was much reduced. The effect of crude anterior pituitary extracts in causing a decreased nitrogen excretion and hypoglycemia in fasted rats has already been reported by Harrison and Long (41).

The results of these first two groups of experiments indicate that some of the metabolic disabilities of the fasting hypophysectomized rat are due to an insufficient supply of cortical hormone. This deficiency results in an inability of the animal to maintain its blood glucose within normal limits by the catabolism of protein. When in addition the animal has already had the supply of insulin reduced by a previous pancreatectomy the elevation of the blood glucose is more marked, presumably by reason of its inability to store some of the newly-formed carbohydrate as liver glycogen. However, in both the hypophysectomized and hypophysectomized-depancreatized ani-

mals following the injection of cortical extract there is an increased catabolism of protein as shown by the increased urinary nitrogen excretion.

The effect of adrenalectomy on the 'diabetogenic' action of anterior pituitary extract. Long and Lukens (75) were unable to exacerbate the diabetes of adrenalectomized-depancreatized cats with anterior pituitary extracts of proved 'diabetogenic' potency. However, Houssay and Biasotti (76) have clearly shown that if adrenalectomized-depancreatized dogs are treated with adequate amounts of cortical extract, the injection of anterior pituitary extract produces a marked elevation of the blood glucose. This difference in results may well be due to the existence of a synergism between these two hormones, such as has been indicated by the recent work of Russell (77).

In the following experiments partially depancreatized rats were used which, prior to adrenalectomy, were either exhibiting glycosuria or devel-

TABLE 15. EFFECT OF APE ON THE GLYCOSURIA OF PARTIALLY DEPANCREATIZED RATS BEFORE AND AFTER ADRENALECTOMY.

The rats were maintained either with NaCl or C.E. after adrenalectomy and the glycosuria is given as the average daily value over the period

Rat No.	Glycosuria, gm./day						Notes
	Before adrenalectomy		After adrenalectomy				
	Control	APE	NaCl	NaCl +APE	C.E.	C.E. +APE	
1	2.63	—	0.08	0.00	—	—	
2	0.00	1.69	0.00	0.00	—	—	
3	0.00	0.84	0.00	0.00	—	—	
11	0.08	0.95	0.00	0.00	—	—	
35	0.00	2.82	0.00	0.00	—	—	
37	0.00	1.27	0.00	0.00	—	—	
61	0.00	1.87	0.00	0.00	0.00	0.00	5.0 cc. C.E. daily
75	4.66	—	—	—	2.60	3.51	5.0 cc. C.E. daily
90A	2.63	3.80	—	—	2.38	2.73	10.0 cc. C.E. daily
102	4.40	—	0.00	0.00	0.00	0.76	3.5 cc. C.E. daily
109	2.20	—	0.00	—	1.24	2.24	5.0 cc. C.E. daily

oped it after the injection of saline extracts of anterior pituitary. Following adrenalectomy these rats were either maintained with sodium chloride or by the oral administration of cortical extract. In either case, after a suitable foreperiod, the same saline extract of anterior pituitary was injected. The results are summarized in table 15 and indicate that unless cortical extract is administered along with the anterior pituitary extract no 'diabetogenic' action of the anterior pituitary extract can be detected. It should be noted that the average values for the period do not give an adequate picture of the response of the glycosuria when anterior pituitary extract and cortical extract are administered simultaneously, since the peak values of the glycosuria are often twice as great as those prevailing prior to the injection of APE.

The failure of the rats maintained on sodium chloride to respond does not seem attributable to cortical insufficiency, if this term is used to connote a moribund condition, since they were eating well and exhibiting none of the signs usually associated with this state. Furthermore, although no glycosuria

occurred, all the injected animals abruptly gained in weight, a gain that was lost following the cessation of treatment.

The nature of the synergism between these hormones is not clear, but since the anterior pituitary is capable of stimulating the adrenal cortex, it would appear that the 'diabetogenic' action of anterior pituitary extract in normal animals requires the operation of two factors, one apparently operating through the adrenal cortex, while the other acts directly on the tissues. Since cortical extract alone is capable of exerting a 'diabetogenic' action in depancreatized or hypophysectomized-depancreatized animals, it would seem that the presence of some cortical hormone is essential for the exhibition of the full 'diabetogenic' action of anterior pituitary extract.

DISCUSSION

The main findings of this work may be considered to be as follows. 1). Adrenalectomized rats or mice, maintained in good health by the oral or intraperitoneal administration of sodium salts and fed glucose, show no marked abnormalities of carbohydrate oxidation or storage. However, when fasted, there is a rapid decline of the liver glycogen and an abnormal fall in muscle glycogen which becomes progressively greater as the fast is continued. The blood glucose also falls below the normal limits and hypoglycemic shock is not infrequent in long-fasted animals in spite of the maintenance of normal electrolyte levels in the serum. Of equal significance, and probably to be associated with the decreased carbohydrate levels, is a definite fall in the rate of protein catabolism as measured by the urinary nitrogen excretion.

2). It is well recognized that the identification of an endocrine gland with a particular function of the organism requires that the administration of suitable extracts of the gland in question should not only restore the deranged function in animals from which the gland has been removed, but should also, when injected into normal animals, produce alterations in function that are in general the reverse of those produced by its removal. Our experiments provide ample evidence along these lines. The injection of cortical extract or certain crystalline steroids isolated from the adrenal cortex into fasted adrenalectomized and normal rats and mice elevates the liver glycogen from ten to forty fold, resulting in levels far beyond those encountered in normal untreated animals. The blood glucose is also significantly increased, but no effects were observed on the muscle glycogen level. In this respect, these findings differ from those of Britton and his colleagues who report that cortical extract increases the muscle glycogen as well as the liver glycogen and blood glucose.

It was apparent from the earlier work of Britton that an absolute increase in the carbohydrate content of the animal occurs under the influence of excess of cortical hormone. The fact that these effects were produced in normal as well as adrenalectomized animals should be sufficient to answer any criticism that the effect of cortical extract on the carbohydrate metabolism of adrenalectomized animals is due to the improvement in their general condition. Furthermore, even in adrenalectomized animals the administration of

an excess of cortical extract raises the carbohydrate levels far beyond those found in normal untreated animals.

3). The source of this newly formed carbohydrate has been sought and it is found that the elevation of the carbohydrate levels of the blood and liver is accompanied by an increased urinary nitrogen excretion of a magnitude that appears sufficient to account for the extra carbohydrate in terms of the increased protein catabolism.

4). The effect of cortical extract on glucose-fed rats indicates that under this circumstance there is a smaller proportion of glucose oxidation and a larger deposition of the absorbed glucose as liver glycogen, while the muscle glycogen level is not increased. The reduction in glucose oxidation may be due to a direct inhibition of its utilization in the tissues or may be a consequence of the retention of an abnormal proportion of the absorbed glucose as liver glycogen. In treated mice fed a high carbohydrate diet the liver glycogen reaches remarkable levels, and in these animals the muscle glycogen is doubled.

5). The evidence gathered from experiments on adrenalectomized and normal rats is substantiated and extended by observations on partially depancreatized rats. In these animals any marked alteration in their ability to form or utilize glucose is reflected by the level of the glycosuria which, by appropriate dietary control, can be held within fairly narrow limits for a long period. It has been shown that adrenalectomy (and also hypophysectomy) is followed by a marked reduction and often a complete disappearance of glycosuria. The administration of the cortical hormone restores the glycosuria to a level depending upon the amount given. Indeed, if very large amounts are administered the subsequent glycosuria will exceed that prevailing prior to adrenalectomy.

It is not only in adrenalectomized-depancreatized animals that glycosuria can be exacerbated by cortical extract. If adequate amounts are administered to fed depancreatized, but otherwise intact, rats glycosuria can be exaggerated. In fasting depancreatized animals that are not excreting glucose this treatment leads to an elevation of the blood glucose and urine nitrogen while glycosuria occasionally reappears. In a few experiments on fed animals no increased nitrogen excretion followed treatment with cortical extract.

6). The results obtained by the study of hypophysectomized rats, whether otherwise intact or depancreatized, must also be considered. The intensive treatment of fasted hypophysectomized rats with cortical extract not only brings about a remarkable improvement in their general condition, but is accompanied by alterations in carbohydrate and protein metabolism similar to those found in normal and adrenalectomized rats. The liver glycogen is increased, but not to the same extent as found in normal rats, while the increase in urine nitrogen is of the same order as found in normal rats. This would indicate that a part of the extra carbohydrate formed has been utilized; an observation that is in keeping with previous experiments on the excessive rate of carbohydrate utilization in these animals. Apparently, part but not all of the disordered carbohydrate metabolism of hypophysectomized

rats can be remedied by the injection of cortical extract. Further observations on the respiratory quotient of treated hypophysectomized rats would throw light on this point, but up to the present these experiments have not been completed.

7). One of the most remarkable effects of hypophysectomy was furnished by the demonstration of Houssay and his colleagues (78) that this operation is followed by an amelioration of total pancreatic diabetes in all species of animals that have been examined. The later observation of Long and Lukens (48) that adrenalectomy has at least a superficially similar effect has raised the question as to how far the results of hypophysectomy are due to the ensuing hypofunction of the adrenal cortex.

In the present experiments it has been shown that in fasted hypophysectomized-depancreatized rats cortical extract exerts a 'diabetogenic' action in the sense that it brings about an elevation of the blood glucose and an increased urinary nitrogen excretion. Such a result might, of course, have been anticipated from the observations on fasted hypophysectomized rats (see part IV).

We may now examine the possibility that a common denominator underlies the action of the cortical hormone on the metabolism of the various preparations in which it has been examined. In the fasting state the injection of this hormone into normal, adrenalectomized, hypophysectomize d, depancreatized or hypophysectomized-depancreatized rats is followed by increased carbohydrate levels in the blood, liver or urine. Furthermore, in each instance there is an unmistakable increase in urine nitrogen while in those experiments in which analyses were made there was found little or no increase in muscle glycogen. The most obvious interpretation of this result would seem to be that in the fasting state, under the influence of the cortical hormone, there occurs an increased rate of protein catabolism. The source of this extra protein has not been determined, but it is of such a magnitude (nearly 200 mg. protein per 100 gm. rat in 12 hours) that it does not seem likely that it all comes from the liver. Such a quantity, however, could be easily released from the muscles. Wherever this source of protein may be it provides a sufficiently large quantity of amino acids to account for the extra liver glycogen and glucose found in the body fluids. Such an explanation would indicate that the cortical hormone stimulates those processes of gluconeogenesis by which the blood glucose is maintained at the expense of the tissue proteins. If this is true, then it might be anticipated that adrenalectomized animals would have difficulty in maintaining an adequate blood glucose level when exposed to circumstances that demand an increased rate of protein catabolism. This has been found to be so since: *a*), adrenalectomized animals exhibit hypoglycemia and a decreased nitrogen excretion when fasted (41); *b*), fasted phloridzinized adrenalectomized rats excrete much smaller quantities of urine glucose and nitrogen than do normal animals (40); *c*), on exposure to low oxygen pressure, fasted normal rats increase their liver glycogen and urine nitrogen, while adrenalectomized animals do not (40); *d*), fasted adrenalectomized animals do not withstand exposure to cold unless treated with cortical extract (79); *e*), fasted adrenalectomized and depancreatized animals

do not excrete the excessive quantities of glucose and nitrogen that are such a characteristic feature of totally depancreatized animals (48).

This hypothesis of the effect of the cortical hormone on protein metabolism does not take into account the alterations in electrolyte (and water) metabolism that are known to be associated with hypo- and hypercortical activity. One of these alterations is, perhaps, capable of explanation in these terms. It is well known from the work of Harrop et al. (80), and Thorn and his colleagues (81), that an increased potassium excretion follows the injection of cortical extract. Since the breakdown of tissue protein is known to liberate potassium in proportion to the extent to which it occurs such a result might be anticipated. The effects on sodium balance and the apparently specific action of the cortical hormone on certain phases of renal function (82) are not at present explicable in terms of this hypothesis. Further work on the metabolic processes furnishing energy for these functions may, however, reveal a connection between these apparently unrelated phenomena.

It appears certain that the adrenal cortical hormones responsible for the effects on carbohydrate metabolism are identical with those that are capable of maintaining both a normal electrolyte balance and the life of adrenalectomized animals. There is, however, in our experience, a quantitative difference between the various crystalline substances isolated from the cortex. Corticosterone and dehydrocorticosterone exert marked effects on both carbohydrate and electrolyte metabolism while desoxycorticosterone and progesterone, while influencing both the duration of life of adrenalectomized animals and their electrolyte balance, have only a comparatively feeble effect on carbohydrate metabolism.

The quantities of cortical extract that we have used to affect carbohydrate metabolism are admittedly large and are probably much greater than those required to maintain the life of adrenalectomized rats. One reason for this may be that the difference between maintenance and optimal amounts of hormone is much greater than would be anticipated. If the amounts of cortical extract used by Ingle, Higgins and Kendall (59), to produce a maximum atrophy of the animals' own adrenal glands are taken as a measure of the daily hormone output of the normal glands then the amounts that we have used are not excessive. Even if this is not the case we have endeavored to produce maximum effects in order that the alterations in carbohydrate levels should be clear and unmistakable.

In conclusion, it may be clearly stated that these observations do not answer many of the debatable points in connection with adrenal cortical function. Nevertheless, they seem to clearly establish the importance of the cortical hormone in the control of both the carbohydrate and protein metabolism of the rat.

The fact that cortical extract alone is able to intensify the diabetes of either partially depancreatized or hypophysectomized-depancreatized rats raises the interesting question as to the relation of these effects to the 'diabetogenic' action of anterior pituitary extract both in normal and in Houssay dogs.

Previous studies on the effect of anterior pituitary extract in adrenalec-

tomized-depancreatized animals have led to conflicting results. Long and Lukens, cf. Long (75), reported that in cats the usual 'diabetogenic' action could not be observed; while Houssay and Biasotti (52) reported positive effects when adrenalectomized-depancreatized toads were implanted with anterior lobes of the same species. In adrenalectomized, but only partially depancreatized, dogs treated with cortical extract, Houssay and Biasotti (76) also observed hyperglycemia after injecting anterior pituitary extract.

The work reported here on rats would indicate, at least in this species, that the presence or absence of cortical extract is the determining factor. The injection of crude saline extracts of the anterior pituitary into adrenalectomized-depancreatized rats does not in our experience increase the glycosuria, even though the same extracts readily produced glycosuria before adrenalectomy. On the other hand, if the rats were maintained on cortical extract after adrenalectomy an increase of glycosuria followed the injection of anterior pituitary extract.

If these observations are correct, it would appear that the cortical hormone alone is able to increase glycosuria, but that all the 'diabetogenic' action of the anterior pituitary is not mediated by the adrenal cortex. The presence of some cortical hormone is, however, necessary for the action of the pituitary factor that acts independently of the adrenals. A similar conclusion as to the synergism between these hormones has been suggested in the recent paper by Russell (77).

SUMMARY

Adrenalectomized mice and rats maintained on sodium salts retain practically normal carbohydrate levels when fed, but on fasting these decline more rapidly than in normal animals. Fasted adrenalectomized animals also excrete less urine nitrogen.

The administration of cortical extract or active crystalline adrenal steroids to either fasted normal or adrenalectomized mice and rats is followed by large increases in liver glycogen and slight hyperglycemia. The muscle glycogen is not affected. At the same time there occur increases in nitrogen excretion of sufficient magnitude to suggest that the increased protein catabolism is the source of this newly formed carbohydrate. In fed animals very large quantities of glycogen accumulate in the liver and under these circumstances the muscle glycogen may also increase.

When the disposition of a known quantity of absorbed glucose is followed in rats it is found that the administration of cortical extract apparently decreases the proportion of glucose oxidized while there is an increased proportion deposited as liver glycogen.

The preparation and care of partially depancreatized rats is described. By the use of these preparations it has been shown (*a*) that adrenalectomy with subsequent maintenance on sodium salts brings about a decreased glucose excretion, (*b*) that the administration of cortical extract, corticosterone, etc. by mouth either exacerbates the glycosuria of animals with intact adrenals or causes its reappearance in adrenalectomized animals maintained on

sodium salts. In the latter case, if sufficient hormone is given, the glycosuria may be raised to levels greater than those existing prior to adrenalectomy. It has also been found that not all the crystalline adrenal steroids possess an equal potency in this regard, desoxycorticosterone having only a feeble effect in comparison to corticosterone and dehydrocorticosterone.

It has been found that not only may the general condition of fasted hypophysectomized rats be greatly improved by the injection of cortical extract, but also the liver glycogen and blood glucose of these animals is increased to supernormal levels. The urine nitrogen excretion is also increased.

Cortical extract is 'diabetogenic' in the fasted hypophysectomized-depancreatized rats in the sense that it increases the blood glucose and urine nitrogen.

Experiments on the 'diabetogenic' effect of anterior pituitary extract in adrenalectomized-depancreatized animals indicate however that not all this activity is mediated by the adrenal cortex, but rather that a synergism exists between these two hormones.

It is suggested that one of the properties of the cortical hormone is a stimulation of protein catabolism, and that the increased carbohydrate levels, nitrogen and potassium excretion following its injection into animals is an expression of this effect.

ACKNOWLEDGMENTS

We are indebted to Dr. K. W. Thompson and Dr. J. A. Russell for performing the hypophysectomies. Miss Hilda B. Ritter has carried out the numerous determinations of urine glucose. We are especially grateful to Dr. E. C. Kendall of the Mayo Clinic and Dr. O. Wintersteiner of the Department of Biochemistry, Columbia University, for sending us samples of the crystalline adrenal steroids. The cortical extract used in these experiments was obtained from the Upjohn Company, and much of it through the kindness of Dr. G. F. Cartland.

This work was supported by grants to one of us (C. N. H. L.) from the Fluid Research Fund, Yale University School of Medicine, and from the Committee on Research in Endocrinology, National Research Council.

Certain parts of this work are taken from a dissertation presented by Benjamin Katzin to the Graduate School, Yale University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1939.

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THE PITUITARY GLAND AND CARBOHYDRATE METABOLISM

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OUR REALIZATION of the important function of the anterior pituitary gland in carbohydrate metabolism undoubtedly dates from the inception of the fundamentally important researches of Houssay and his colleagues. Before Houssay's work, clinical and experimental observations had suggested that the posterior lobe of the hypophysis was of more importance in carbohydrate metabolism than the anterior part of that gland, but the results of Houssay and his collaborators stressed the predominant rôle of the anterior lobe in this connection. Although the significance of the posterior pituitary in carbohydrate metabolism is by no means certain we are now sure that the anterior lobe is of great importance therein.

The primary points established by Houssay and his colleagues were the following. (*a*) Removal of the pituitary increases the sensitivity to insulin of the normal animal (1) and diminishes the intensity of the diabetic condition of a depancreatized animal (2, 3). (*b*) Treatment of a normal or of a hypophysectomized animal with preparations from the anterior pituitary diminishes its sensitivity to the hypoglycemic action of insulin, while similar treatment of a hypophysectomized-depancreatized animal also increases the severity of its diabetic state (3, 4). (*c*) The administration of a suitable anterior pituitary extract to normal animals can induce a diabetic condition (5). Although the third point was a natural corollary of the previous findings, Houssay's observation in this connection was independently anticipated by publications from two other laboratories, by Evans, Meyer, Simpson and Reichert (6), and by Baumann and Marine (7). In the experiments of Evans and his colleagues a diabetic condition was observed in 2 dogs, but not in their respective treated litter mates, 8 to 9 months after the daily administration of a preparation of the pituitary growth hormone had begun. When treatment was stopped the glycosuria persisted for 2 months in one instance, disappearing at the end of this time. The other animal, which was in a very bad condition at the time glycosuria was detected, was found still to have sugar in the urine 4 months after the cessation of treatment, and although at the time of this animal's death the polyuria had diminished, the animal was then still passing sugar (8). This demonstration of the diabetogenic action of an anterior pituitary extract naturally aroused great interest, but unlike Evans and his colleagues, other authors observed the induction of a diabetic condition within a few days of the initiation of daily injection of pituitary extract, and the disappearance of the symptoms within a few days of cessation of treatment.

These primary observations of Houssay and others showed that the secretions of the anterior pituitary profoundly influence carbohydrate

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THE PITUITARY GLAND AND CARBOHYDRATE METABOLISM

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From the National Institute for Medical Research

HAMPSTEAD, LONDON, ENGLAND

OUR REALIZATION of the important function of the anterior pituitary gland in carbohydrate metabolism undoubtedly dates from the inception of the fundamentally important researches of Houssay and his colleagues. Before Houssay's work, clinical and experimental observations had suggested that the posterior lobe of the hypophysis was of more importance in carbohydrate metabolism than the anterior part of that gland, but the results of Houssay and his collaborators stressed the predominant rôle of the anterior lobe in this connection. Although the significance of the posterior pituitary in carbohydrate metabolism is by no means certain we are now sure that the anterior lobe is of great importance therein.

The primary points established by Houssay and his colleagues were the following. (*a*) Removal of the pituitary increases the sensitivity to insulin of the normal animal (1) and diminishes the intensity of the diabetic condition of a depancreatized animal (2, 3). (*b*) Treatment of a normal or of a hypophysectomized animal with preparations from the anterior pituitary diminishes its sensitivity to the hypoglycemic action of insulin, while similar treatment of a hypophysectomized-depancreatized animal also increases the severity of its diabetic state (3, 4). (*c*) The administration of a suitable anterior pituitary extract to normal animals can induce a diabetic condition (5). Although the third point was a natural corollary of the previous findings, Houssay's observation in this connection was independently anticipated by publications from two other laboratories, by Evans, Meyer, Simpson and Reichert (6), and by Baumann and Marine (7). In the experiments of Evans and his colleagues a diabetic condition was observed in 2 dogs, but not in their respective treated litter mates, 8 to 9 months after the daily administration of a preparation of the pituitary growth hormone had begun. When treatment was stopped the glycosuria persisted for 2 months in one instance, disappearing at the end of this time. The other animal, which was in a very bad condition at the time glycosuria was detected, was found still to have sugar in the urine 4 months after the cessation of treatment, and although at the time of this animal's death the polyuria had diminished, the animal was then still passing sugar (8). This demonstration of the diabetogenic action of an anterior pituitary extract naturally aroused great interest, but unlike Evans and his colleagues, other authors observed the induction of a diabetic condition within a few days of the initiation of daily injection of pituitary extract, and the disappearance of the symptoms within a few days of cessation of treatment.

These primary observations of Houssay and others showed that the secretions of the anterior pituitary profoundly influence carbohydrate

metabolism, stimulating those processes which, driven to excess, lead to diabetes, As the production of a diabetic condition is a complex phenomenon involving a derangement of more than one process of normal metabolism, it is pertinent to inquire how many of the alleged anterior lobe hormones are concerned in the diabetogenic action of anterior pituitary extracts. Although no final or even definite answer can be given to such a question, we can discuss under separate headings, a number of different effects of anterior pituitary extracts, any or all of which may be concerned in the induction of a diabetic condition. In each case the possible identity of the active substance will be considered.

The Anti-insulin Action of Anterior Lobe Extracts

As was first demonstrated by Houssay and Potick (2) and Benedetto (4), and adequately confirmed by later investigators, treatment with anterior lobe extracts can induce, in normal or hypophysectomized animals, insensitivity to the action of a subsequently injected test dose of insulin. Evidence has accumulated to show that the substance inducing insulin insensitivity (the name 'glycotropic substance' has been suggested for this active principle) is not itself diabetogenic, and that a high degree of insulin insensitivity may be induced by treatment with extracts which leave the resting blood sugar level virtually unchanged (9).

There is evidence that the glycotropic substance is not identical with prolactin (9), although preparations of this substance are often contaminated with it. Collip (10) found that preparations of his heat-stable metabolism-stimulating principle possessed glycotropic activity, and the heat stability of solutions of the glycotropic substance has been confirmed (9). In our experience solutions of the anterior pituitary ketogenic principle are much less heat-stable than those of the glycotropic substance (11). Collip (10) has suggested that his metabolism-stimulating principle may be identical with the melanophore-expanding substance (frog) from the pars intermedia of the pituitary; we have obtained evidence that the melanophore-expanding substance and the glycotropic substance are not identical. Treatment of normal fasting animals with anterior pituitary preparations possessing glycotropic activity, results in the maintenance of abnormally large stores of liver and muscle glycogen during a short period of fasting (12, 13). The evidence at present available suggests that the glycotropic substance is not identical with prolactin, nor with the thyrotropic, gonadotropic (9), ketogenic or melanophore-expanding substances. As the glycotropic substance is not itself diabetogenic in normal animals, it cannot be identical with the diabetogenic principle of the anterior pituitary, although it may conceivably be a constituent of a diabetogenic complex. The glycotropic substance has so far not been differentiated from a number of other pituitary substances, and in particular from the 'glycostatic' (14) substance.

The Ketogenic Action of Anterior Lobe Extracts

Since, in 1930, Burn and Ling (15) observed a ketogenic action of anterior pituitary extracts there has been much interest in the possible relation of this

action of pituitary extracts to that exerted on carbohydrate metabolism.

Shipley and Long (16) have suggested that the ketogenic action of anterior pituitary preparations may not be due to the action of a separate ketogenic principle but may result from the action of such extracts in diminishing the catabolism of carbohydrate and of protein.

The ketogenic factor can be differentiated from the glycotropic substance by its greater heat lability in solution (11). It is present in preparations of the diabetogenic principle. Shipley and Long (16) make the interesting suggestion that the ketogenic factor and the growth and diabetogenic principles may be identical.

The Diabetogenic Action of Anterior Pituitary Extracts

The diabetogenic action of crude anterior pituitary extracts, characterized by ability to induce the appearance of the symptoms of diabetes in normal animals, is lost if the glands are allowed to remain at room temperature for some hours. On the other hand, the administration of heat-stable anterior lobe substances to hypophysectomized-depancreatized animals may be followed by a substantial increase in glycosuria (cf. Long, 17). It is therefore possible that the principle which is diabetogenic in the normal animals consists of at least two parts, one of which is heat labile and the other which is heat stable (17, 18), the latter having an extra-pancreatic action. Houssay and Foglia (19) have adduced evidence that in dogs made diabetic by treatment with anterior pituitary extract, the rate of secretion of insulin by the pancreas is diminished. This diminution in secretion may be the result of partial exhaustion of the insulin-secretory mechanism brought about by the maintenance of a high blood sugar level by extra-pancreatic means, but it is possible that one action of the diabetogenic principle is directly to diminish the rate of secretion of insulin by pancreatic islet cells. Because of this possibility, and taking into account the dual action of diabetogenic pituitary extracts on the pancreas and on the extra-pancreatic tissues, we have preferred to use normal animals as test objects for the diabetogenic action of anterior pituitary extracts, rather than hypophysectomized-depancreatized animals or animals partially depancreatized but with the pituitary gland intact. In the following discussion the term 'diabetogenic' will be used to describe extracts which, on repeated administration to normal dogs, induce hyperglycemia, glycosuria and ketonuria.

When the same amount of anterior lobe extract is injected daily into a dog for a period of a week or more, according to our experience a latent period of 3 to 5 days occurs before the blood sugar rises to a diabetic level and glycosuria is observed; this is in agreement with the previous findings of Houssay and his colleagues (20). We have observed that the sensitivity of the animal to the hypoglycemic action of insulin is substantially diminished during this latent period, even though the fasting blood sugar level is not significantly increased. When the daily injections are continued beyond the latent period, glycosuria, ketonuria, polyuria, polydipsia and hyperlipemia are observed, but in our experiments the body weight tends to rise rather

than to fall. We have been able to confirm the observations (21) that normal liver glycogen levels may be maintained during this period of diabetes. In spite of the continued daily administration of the same amount of anterior pituitary extract, the blood sugar may fall to a normal level and the diabetic condition disappear; nevertheless, the animal remains relatively insensitive to the hypoglycemic action of administered insulin. If the amount of extract injected daily is increased, the diabetic condition may reappear but despite the continuation of treatment, the glycosuria again subsides. If, however, a sufficiently large amount of extract is administered each day the animal is unable to develop refractoriness to the diabetogenic action, and in such cases the diabetic condition may not only fail to disappear when the daily injections of the same amount of extract are continued, but may continue indefinitely after the cessation of treatment with the extract (22). In this way a definitive, permanently diabetic condition can be produced by only a few weeks' treatment with anterior pituitary extract. These results have been confirmed by other workers (23, 24, 25). The development of refractoriness to the diabetogenic action of the extract can be avoided if the amount injected daily is increased every few days. A permanently diabetic condition has thus been established as the result of as little as 11 days treatment with anterior pituitary extract.

The nature of the permanent diabetes thus produced in dogs cannot be discussed in detail here. It is sufficient to mention the following points which have been observed largely as the result of investigations in collaboration with Mr. H. P. Marks. (*a*) In our experience the diabetic condition, once established, shows no sign of spontaneous remission; on the other hand, the ketonuria may rise somewhat over a period of a year or more after the cessation of treatment with anterior pituitary extract. (*b*) The animals are able to survive for long periods without insulin therapy in spite of the heavy glycosuria, and if sufficient food is given the loss of body weight is small or even negligible. Nevertheless, if insulin is suddenly withdrawn after a period of therapy the animal may die in a state resembling diabetic coma. (*c*) Although these dogs are able to survive for a long time without insulin, some of them may require substantially more insulin to control glycosuria than do depancreatized dogs under similar conditions, although the same amount of food be absorbed by both types of diabetic animal. (*d*) When these animals receive a diet consisting of beef suet only, their glycosuria and ketonuria are both substantially diminished. One animal lived for some weeks on such a diet, and as the result of this régime glycosuria was abolished, sugar tolerance increased and ketonuria almost abolished. Glycosuria and ketonuria both returned when this animal subsequently received a diet of raw meat. (*e*) The islets of Langerhans of the pancreas show changes ranging from depletion of the cytoplasmic granules of the beta cells to extensive hyaline replacement of the cellular tissue (26). These changes are sufficient, in many cases, to account for the persistence of a severely diabetic condition.

Fractionation of the diabetogenic substance of ox anterior lobe extracts has been carried out at 0° C. From these investigations it appears that the

diabetogenic substance is not identical with prolactin, nor with the thyrotropic, gonadotropic or glycotropic substances of the anterior lobe, nor with the melanophore-expanding principle of the pars intermedia. In our experience it is not dialyzable. It has not been differentiated from the ketogenic, glycostatic (14), or adrenotropic substances. Collip (10) has shown that preparations of his metabolism-stimulating factor are not diabetogenic in the intact dog. The relationship of the growth and diabetogenic principles is discussed below.

The Pancreotropic Action of Anterior Pituitary Extracts

In 1933, Anselmino, Herold and Hoffman (27) stated that in rats frequent injections of anterior lobe extract produce, in a few days, an increase in the size and number of the islets of Langerhans of the pancreas. Richardson and Young (28) were unable to confirm these findings under the conditions defined by the German investigators, but nevertheless were able to show, using a quantitative method for the assay of islet tissue, that daily treatment with crude anterior lobe extracts for 2 weeks doubled the amount of islet tissue in the pancreas of the rat. Recently Marks and Young (29) have shown that the insulin content of the rat pancreas is greatly increased under the same conditions.

It is possible that such a pancreotropic action of anterior pituitary extracts explains the development of refractoriness to their diabetogenic action observed in the dog (see above). In agreement with this idea Richardson and Young (26) observed remarkable mitotic activity in the pancreatic islets of dogs which had become refractory to the diabetogenic action of a crude pituitary extract. It should be mentioned that in our experience the Wistar strain of rat fails to exhibit a diabetic condition as the result of treatment with anterior pituitary extracts which are effective in the dog. This insensitivity to the diabetogenic action of the extract in the rat may well be due to the relatively rapid response of the rat islet-tissue to the pancreotropic action of the extract. It seems probable, therefore, that the pancreotropic action of the extract is not merely a compensatory response to its diabetogenic action.

The Question of a Separate 'Diabetogenic' Principle

As the pituitary gland obviously does not secrete a principle whose express function is to produce a pathological condition, the term 'diabetogenic hormone' is inappropriate. The different actions of anterior pituitary extracts which we have considered, viz., inhibition of insulin action; inhibition of insulin secretion; stimulation of glycogen accumulation in liver and muscles, together with depression of carbohydrate oxidation demonstrated by Russell, Bennett and others; would all tend to modify metabolism in such a manner as to intensify an already existing diabetes, or to provoke the appearance of such a state if it did not already exist. On the other hand, an enhanced secretion of insulin by the pancreas might overcome such a tendency. We have observed that diabetogenic preparations from the anterior lobe usually possess growth promoting activity, and it seems possible that the growth

substance and the diabetogenic principle may be closely related if not identical, the diabetogenic action of an extract becoming manifest in those cases in which the pancreatic islets are unable to secrete sufficient insulin to overcome the diabetogenic influence of the extra-pancreatic action of the extract. The recent conclusions of Mirsky and Swadesh (30) are of great interest in this connection. These authors believe that the anterior lobe exerts two types of action on protein metabolism in the normal animal: (*a*) a direct action on the muscles stimulating protein catabolism; (*b*) an indirect action through the pancreas, stimulating protein anabolism. They suggest that the influence of the pituitary gland on growth may be dependent on its pancreotropic function.

It seems possible that the growth-promoting action of anterior lobe extracts may be contingent on the ability of the pancreas to secrete sufficient insulin to induce nitrogen retention and promote carbohydrate oxidation. If the pancreas is able to do so, as presumably is the case in the rat, the extracts exert a growth-promoting action rather than a diabetogenic one. If the available insulin is unable to prevent protein breakdown, then sugar may be excreted and the extract may thus exhibit a diabetogenic action. The diabetogenic action may, however, disappear, if, as may be the case with the dog, the pancreatic-islet tissue is eventually stimulated by the pancreotropic action of the extract to such a degree as to produce insulin sufficient to overcome the extra-pancreatic diabetogenic action of the extract. Until the identity of the pancreotropic substance is clear, it would be unwise to assume that the growth-promoting agent in anterior lobe extracts is identical in every respect with that which has been named the diabetogenic substance. Whatever may be the case there is of course no need to assume that the diabetogenic principle consists of one substance only; it may well consist of a complex in which a number of active principles are concerned.

If the diabetogenic substance is closely related to, or identical with, the growth-promoting principle of the anterior pituitary lobe, then the experiments of Evans and his colleagues, in which the diabetogenic action of anterior lobe extracts was first demonstrated in normal animals with growth-promoting preparations, may receive a new significance.

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ASSOCIATION NOTICE

ANNOUNCEMENT OF THE ANNUAL MEETING

THE TWENTY-FOURTH ANNUAL MEETING of the Association for the Study of Internal Secretions will be held in New York City on June 10th and 11th, 1940. The Hotel Roosevelt at Madison Avenue and 45th Street will be the headquarters for registration and for the scientific and business sessions.

The Chairman of the Local Committee is Dr. E. T. Engle, 630 West 168th St., New York City.

Instructions regarding the reservation of hotel accomodations will be given all members at the time of the mailing of the annual statements.

PRESENTATION OF PAPERS

Members of the Association and other investigators who wish to present papers will please note the following regulations regarding the program for the coming meetings.

1. The title of the paper, and a comprehensive abstract in *quadruplicate*, must reach the President, Dr. Philip E. Smith, 630 West 168th Street, New York, N. Y., not later than March 1, 1940.

2. Non-members who present papers will appear on the program "by invitation." Non-members are therefore requested to have their titles and abstracts introduced by a member.

3. The abstracts of papers will be considered by the program committee and the final program will be announced about April 15, 1940.

4. Papers will be limited to ten minutes for presentation and five minutes for discussion.

5. All papers presented before the Association or read by title are to be submitted, in completed manuscript form, to ENDOCRINOLOGY, either before or immediately after the meeting. It is to be understood, however, that acceptance of a paper for the program does not obligate the Journal to its acceptance for publication.

You are invited to bring this announcement to the attention of your colleagues and associates who have endocrine research in progress.

NOMINATIONS OF OFFICERS

The By-Laws of the Association provide that nominations for all elective offices shall be made by a Nominating Committee and forwarded to the Secretary at least sixty days before the annual meeting. The Nominating Committee for the current year, appointed by the President and accepted by the Council, is as follows.

Dr. David P. Barr, *Chairman*, 600 South Kingshighway, St. Louis, Mo.
Dr. E. C. Kendall, Mayo Clinic, Rochester, Minn.
Dr. W. O. Nelson, 1512 St. Antoine St., Detroit, Mich.

The By-Laws also provide (Article V, Section 2) that "Any member of the Association may submit nominations to the Nominating Committee for its consideration." Any nominations which members desire to make should be sent to Dr. David P. Barr by March 1, 1940.

The terms of the following officers expire at the time of the annual meeting in New York in 1940.

President-Elect	E. L. Sevringhaus
Vice-President	Max Goldzieher
Secretary-Treasurer	E. Kost Shelton

Council Members for 3 years	Publication Board for 3 years
J. P. Pratt	Edgar Allen
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E. C. Hamblen	

THE E. R. SQUIBB & SONS AWARD

At the Annual Meeting in 1939 the Council directed that the President should appoint a committee to set up the permanent mechanism for making the award. This committee, consisting of Dr. E. L. Sevringhaus, *Chairman*, Dr. F. A. Hartman and Dr. David P. Barr, has drawn up the following regulations for the administration of the E. R. Squibb & Sons Award.

1. In order to encourage investigation in endocrinology, E. R. Squibb & Sons have established an annual award of one thousand dollars to be given through the medium of the Association for the Study of Internal Secretions. The Association through a Committee of Judges selected from its membership, will name the recipient of the award; and the formal presentation will be made part of the annual program.

2. The award will be given to the investigator or investigators in the United States or Canada for one of the best contributions to endocrinology. If in the committee's judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons.

3. A Committee of Judges consisting of five members of the Association will be appointed by the President to recommend to the Council a recipient for the award. The members of this committee shall hold office for five years, one new member being appointed each year. The first year the appointments shall run for one, two, three, four and five years respectively. Nominations for the award may be sent to the Secretary by members of the Associaton not later than January 15 of the year in which the award is to be made.

4. The name of the nominee selected by the Committee of Judges should be sent to the Secretary of the Associaton two months before the annual meeting. This nomination will then be submitted to the Council for ratification and thereafter the nominee will be notified in order that he may make plans for being present at the annual meeting. The name of the nominee will be kept confidential by all concerned until the announcement is made at the annual dinner, at which time the award will be bestowed by the President of the Association. The recipient will be expected to make a brief response.

5. It is desirable that the award be made annually. However, if in any year no suitable candidate is found the Council retains the privilege of witholding the award for that year.

6. In addition to the purse provided by E. R. Squibb & Sons the recipient will be presented with a simple scroll as a memento of the occasion, the cost to be defrayed by the Association.

In accord with paragraph 3 of the above regulations the President has appointed the following Committee of Judges: Dr. E. A. Doisy, for five years; Dr. E. C. Hamblen, for four years; Dr. E. T. Engle, for three years; Dr. F. C. Koch, for two years and Dr. E. Kost Shelton, for one year. Dr. Doisy was named Chairman for the first year. This Committee has made the following announcement.

Announcement of the Committee of Judges

In order that members may have more time to make their nominations for the first E. R. Squibb & Sons Award, the Committee will accept nominations until March 1, 1940.

Each member has the privilege of making *one* nomination. A nomination should be accompanied by a statement of the importance of the nominee's contributions in endocrinology and by a bibliography of the nominee's most important publications in endocrinology. Five copies should be sent to the Secretary, Dr. E. Kost Shelton, 921 Westwood Boulevard, Los Angeles, California, who will transmit a copy to each member of the Committee.

Since the main qualification for the Award is the importance of the nominee's contributions to endocrinology, it seems undesirable for the Committee to make any definite restrictions regarding age of the nominees or the time and place of publication of the contributions.

Philip E. Smith, *President*
E. Kost Shelton, *Secretary*

CURRENT ENDOCRINE LITERATURE

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ADVANCE ABSTRACTS

BABKIN, B. P., O. KOMAROV AND S. A. KOMAROV. The effect of activated ergosterol and of parathyroid hormone on gastric secretion in the dog. *To appear in this Journal.*

The effect of irradiated ergosterol on gastric secretion was studied in 2 Pavlov-pouch dogs. Ergosterol, administered continuously for some time in daily doses sufficient to raise the normal serum Ca eventually to 15.0 mg. % or higher, caused a definite inhibition of the nervous phase of the gastric secretions provoked by meals of bread, milk and meat respectively. The inhibition developed gradually in proportion to the degree of hypercalcemia produced. In every case the volume, acidity and peptic power of the secretion, and especially the total output of pepsin, were below normal. These effects were observed regularly even when no general toxic effects of hypercalcemia were detectable. Irradiated ergosterol had but a slight effect, if any, on the chemical phase of gastric secretion evoked by the above-mentioned test-meals and no effect whatever on the gastric response to subcutaneous injection of histamine. Its inhibitory effect on the nervous phase persisted long after its administration had been discontinued, subsiding gradually as the blood Ca returned to normal. However, some degree of inhibition was noticeable for several weeks after the blood Ca became normal. After intravenous injection of 0.1 gm. calcium lactate (13 mg. Ca) per kg. of body weight, the nervous phase of the gastric secretion was markedly inhibited for several hours. Some studies on the effect of daily administration of parathormone in a dog with esophagotomy and a gastric fistula showed that the volume of gastric secretion provoked by sham-feeding and the total output of acid and of pepsin were diminished.

BURRILL, M. W., AND R. R. GREENE. Further studies on the andromimetic function of the immature male rat adrenal. *To appear in this Journal.*

The functional status of the immature male ventral prostate was studied in the intact, the castrated, the adrenalectomized and the adrenalectomized-castrated rat at different age levels. Using gross weight and cytological findings as criteria, the ventral prostates were found to be partially maintained in castrated rats at 26 and 31 days of age, but not at 36 and 41 days of age. Complete castration atrophy was found in the prostates of adrenalectomized-castrates at all age levels investigated. In the presence of the testes, adrenalectomy alone did not modify the ventral prostates at any of these ages. From these data it was concluded that, in the absence of the testes, the adrenals have an andromimetic capacity in the younger immature rat (26 and 31 days old). This capacity was lost in the older immature animals (36 and 41 days old).

ELDEN, C. A., AND M-D. F. NUTTING. A method of preparation of the gonadotropic hormone from the anterior pituitary gland of the sow. *To appear in this Journal.*

The gonadotropic hormone from the anterior lobe of the pituitary of the sow can be prepared by precipitation with dioxane at a PH 7.1 to 7.3. One unit of the hormone thus prepared was found to weigh 0.00056 gm.

FREED, S. C. The mechanism and treatment of premenstrual distress with ammonium chloride. *To appear in this Journal.*

Premenstrual distress or tension may include one or several of the following: headache, nausea,

bloating of the abdomen, emotional disturbances of many kinds and edema. The development of these symptoms is considered as resulting from sodium ion retention of various tissues of the body with subsequent increase in extra-cellular fluid. The steroids of the ovary are probably responsible for this reaction. Withdrawal of excess extracellular fluid by the use of ammonium chloride in the second two weeks of the menstrual cycle is followed uniformly, by relief from premenstrual discomfort.

HELLER, C. G. Metabolism of the estrogens. The effect of liver and uterus upon estrone, estradiol and estriol. *To appear in this Journal.*

In vitro studies are reported on the effect of tissue slices and tissue mince upon estradiol, estrone and estriol as judged by their biological activity. Estradiol is inactivated by liver and kidney and not by the other tissues studied. This inactivation of estradiol is, in all probability, not due to conjugation or conversion to a less active form, but due to enzymatic destruction of an oxidative nature. This is indicated by the relative inactivity of the treated estradiol, the inability of hydrolysis procedures to increase activity and the effect of tissue poisons upon inhibiting the estradiol-destroying enzyme system. Estrone is increased 20 times in potency by incubation with minced uterine. Spleen, lung, heart and kidney tissues also caused estrone to be converted to a more active substance. The suggestion is made that this conversion is accomplished by enzymatic reduction of estrone to estradiol. Liver tissue, which has been found normally to destroy estrone, when poisoned with NaCN causes an increase in estrone potency. Since NaCN also inhibits the estradiol-destroying system in liver it is suggested that liver contains both an estrone reducing enzyme system which converts estrone to estradiol, and an estradiol-destroying system which inactivates the estradiol so formed. All respiring tissues studied caused the hydrolysis of estradiol glucoside and estradiol benzoate. An explanation is offered for the better results obtained with orally administered estriol than with orally administered estradiol and estrone, since estriol is only mildly affected by liver tissue in contrast to the great destruction of estrone and estradiol.

HETHERINGTON, A. W., AND A. WEIL. The lipoid, calcium, phosphorus, and iron content of rats with hypothalamic and hypophyseal damage. *To appear in this Journal.*

The bodies of 4 pathologically obese rats, one completely hypophysectomized rat, and one partially hypophysectomized rat, all females, were separated into their lipoid and dried non-lipoid fractions. Two of the 4 fat animals had been produced by injecting chromic acid into the hypophyses (the acid having caused damage to the neighboring hypothalamus) and 2 by placing electrolytic lesions in the hypothalamus with the Horseley-Clarke instrument. It was found that the obese rats contained from 2 to 6 times as much fat as their litter-mate controls, but the dwarfs contained approximately the same proportion as the normals. Chemical analysis of the lipoid and defatted fractions for phosphorus, and of the latter portion for calcium showed that the supply of both elements had been considerably depleted in the bodies of the fat rats. Calcium, but not phosphorus, was diminished in percentage amount in the dwarfs. Iron values were irregularly lower in the obese animals. It would seem that besides the obviously disordered fat metabolism in these cases of adiposity there is a more widespread upset of the body's physiological economy which takes in the calcium, phosphorus and iron exchanges, and possibly other factors as well.

INGLE, D. J. The work performance of adrenalectomized rats treated with corticosterone and chemically related compounds. *To appear in this Journal.*

When the gastrocnemius muscle of the normal anesthetized rat is weighted with 100 gm. and stimulated to contract 3 times per second the muscle continues to work for several days. When rats are subjected to work immediately following adrenalectomy muscular responsiveness is lost within a few hours in the untreated animals whereas similar animals treated with cortin can work for several days in a nearly normal manner. In these studies the property of maintaining the capacity of adrenalectomized rats to work was found to be possessed by corticosterone, desoxycorticosterone, and 17-oxy-corticosterone, 17-oxy-11-dehydrocorticosterone, and 11-dehydrocorticosterone. Little or no effect was obtained by the administration of desoxycorticosterone acetate. A wide discrepancy was noted between the apparent activities of these compounds as judged by the effect on work in this test and the life-maintenance activity as determined by others. This discrepancy may be due to either a qualitative difference in the biologic effects of these compounds or to differences in their utilization. Reduction of the ethylenic bond (carbons 4–5) in 17-oxy-11-dehydrocorticosterone destroyed its effect on work. Progesterone, ethinyl testosterone, testosterone, testosterone proprionate, estradiol benzoate and stilbestrol were without effect upon work performance.

KLEINER, I. S. The relative effects of progesterone and testosterone proprionate upon ovipositor lengthening of the female bitterling. *To appear in this Journal.*

De Wit's statement that progesterone appears to be about 125 times as active as testosterone proprionate in its effect upon the lengthening of

the ovipositor of the female bitterling is subjected to criticism and the experiments were repeated. Although progesterone does have an effect in considerably greater dilution, this effect is relatively slight. Neither in de Wit's report nor in the author's experience does this hormone have an effect equivalent to that of a "bitterling unit" of male hormone. Moreover, the maximum progesterone action takes place during the first 6 to 8 hours while that of testosterone is observed only in 24 to 72 hours.

KOBER, S. Pregnancy diagnosis in mares by colorimetric estimation of estrogens. *To appear in this Journal.*

The estrogen content in the urine of 3.597 covered mares has been determined using the author's colorimetric estimation. If the results are interpreted in view of a pregnancy test, the total percentage error over all the material lies between 0.61 and 0.33, the error being smaller in the group that foaled than in the group that did not.

KUZELL, W. C., AND W. C. CUTTING. Pituitary mitotic changes after the administration of estrogen and after ovariectomy. *To appear in this Journal.*

In rats injected with estrogen, or ovariectomized, evidence of pituitary growth, as demonstrated by the numbers of mitoses after colchicine injection, was as follows. 1. Ovariectomized rats showed no consistent evidence of pituitary growth. 2. Estrogen injected rats showed evidence of a considerable and prolonged pituitary growth.

LEE, M. O., AND W. FREEMAN. Liver growth in rats treated with anterior pituitary growth hormone. *To appear in this Journal.*

Estimations were made of the number of hepatic cells in the livers of 23 normal control rats, 23 litter mates treated with AP growth extract for 1 to 16 days and kept on a paired-feeding regimen, and 8 litter mates treated with growth hormone but allowed food ad libitum. The average liver weight, the ratio of liver weight to body weight and the total number of hepatic cells were significantly greater in the treated animals than in the controls. The number of hepatic cells per gram of fresh liver was essentially the same in the 2 groups. Histological study indicated that there were no differences between the 2 groups in the proportion of the structural elements other than hepatic cells. In ad libitum-fed treated rats the increase in number of hepatic cells was the same as in the treated mates with restricted food consumption, but the increase in mass of the liver was greater. The nitrogen content per gram of liver was the same in all groups. It is concluded that under the influence of the growth hormone the liver responds with a true hyperplasia, with all of the structural components participating equally.

MARLOW, H. W., AND D. RICHERT. Estrogens of the fowl. *To appear in this Journal.*

Exhaustive extraction and assay of hen ovaries, immature ova, follicular membrane and fresh egg yolk disclosed that all had some estrogenic activity. Hens' ovaries gave 37 R.U./kg., immature ova 44 R.U./kg., follicular membrane 166 R.U./kg. and fresh egg yolks 5 R.U./kg.

MAZER, M., AND C. MAZER. The effects of testosterone proprionate on the ovariectomized mature rat. *To appear in this Journal.*

The experiment described was designed to determine the effect of length of treatment with testosterone proptionate on the ovariectomized rat. After ovariectomy and estrogen priming groups of rats were given daily injections of 2 mg. of testosterone proprionate in sesame oil. Control rats were treated similarly except that sesame oil alone was administered. By killing groups of animals after 3, 8, 15, 23 and 29 days of treatment, it was found that the estrogenic effect of testosterone proprionate on the uterus appeared early and was progressive throughout the duration of treatment. The progestin effect, on the other hand, did not appear before the 8th day of treatment and was not complete before the 15th day. Longer treatment maintained the progestational appearance of the endometrium but produced cystic changes therein. Despite vaginal growth, cornification of the epithelium was not noted. Instead, marked mucification of the vaginal epithelium occurred. The preputial glands were enormously enlarged as a result of testosterone treatment. This seems to be a distinctive effect of the substance. The adrenal glands were smaller in the treated than in the control animals, presumably through inhibition of pituitary function.

MOFFAT, W. M. The effect of APL sex hormone on the blood picture in man. *To appear in this Journal.*

Gonadotropic hormone extracted from pregnancy urine (follutein) was administered intramuscularly to 38 individuals, 8 males and 30 females, in doses ranging from 100 to 400 R.U. Complete blood counts were made in the afternoon and again the next morning immediately preceding injection, and then at 2, 4, 6, 8, 15 and 24 hours following injection. A leucocytosis appeared at 4 hours, reached its greatest height at 6 or 8 hours, and then diminished but did not disappear at 24 hours. The degree of leucocytosis was roughly proportional to the amount of follutein given. There was an increase in the absolute number of white cells of all types with a relative neu trophilia. Following daily repeated doses the leuco-

cytosis gradually diminished and eventually disappeared but after an interval of 2 to 3 weeks could again be elicited. The leucocytosis did not seem to be a protein or toxic reaction. The same effects were observed in both sexes. There was no change in the red cell count nor in hemoglobin.

PARKER, F., JR., AND B. TENNEY, JR. Further studies of the hormone content of human tissues in pregnancy. *To appear in this Journal.*

Additional work is presented on the extraction of human tissues in pregnancy. The amount of P.U. in maternal and fetal organs as well as body fluids is given in tabular form. In contrast to a previous paper in which the estrogens were found in large amounts in fetal and maternal organs, this paper demonstrates that P.U. is confined to placenta, maternal blood and urine. It is not found in appreciable amount in the fetal blood, organs or amniotic fluid. It is also not found in the maternal organs.

SHERWOOD, T. C. The effect of stilbestrol on the basal metabolism of experimental hyperthyroid rats. *To appear in this Journal.*

Sixteen rats were ovariectomized and thyroidectomized after the normal basal metabolism had been established. The metabolic rate was determined at 28° C. and the data calculated in calories/sq. m. of body surface. When the hypothyroid level had been determined, desiccated thyroid was administered to each animal for a period of 3 days, the daily dose being 1000 mg./kg. of body weight. Metabolism tests were then run daily until the hypothyroid level had again been established. Nine of the animals were given 3 daily injections of 10 mg. of stilbestrol immediately following the cessation of thyroid feeding. The hypothyroid basal metabolic level was obtained in approximately one-half the time when stilbestrol injections were given to experimental hyperthyroid rats immediately following the cessation of thyroid feeding.

STARKEY, W. F., AND J. H. LEATHEM. Effect of a normal male urine extract on immature female cats. *To appear in this Journal.*

Fifteen of 18 immature female cats, 5 to 13 weeks of age, responded to an extract of normal male urine, 'prospermin.' Ovarian weight increased as much as 8-fold over the controls. After 11 days treatment, only follicle stimulation was observed but extended treatment resulted in the formation of corpora lutea atretica, although the total dose was fairly comparable in some cases. In most cats, both ovaries either did or did not respond, but in 3 animals only the right ovary responded. Furthermore, considerable variation of the degree of stimulation resulted among litter-mate cats that had received the same hormonal treatment. The capability of the individual ovary to react at the time of treatment appeared to determine the response rather than the age of the cat. An estrous or pseudopregnant endometrium developed in association with the state of the ovary.

SUDDS, M. U. N. The cell contents of the cortex of the suprarenal gland. *To appear in this Journal.*

Owing to the fact that discrete fuchsinophil granules were discovered in 3 malignant cortical suprarenal tumors in females manifesting virilism, an investigation was carried out on suitable postmortem material collected over 3 years at The Royal Free Hospital, London, to ascertain whether these granules were associated with sex hormones. Discrete fuchsinophil granules were found to be present in 24% of adult male suprarenals examined and in 28% of female suprarenals. The ages ranged between 24 years and 72 years in females and 27 and 75 in males. The increase in the percentage showing granules with advancing age is steady in females, and the apparent peak in the male age group 40 to 50, with the subsequent drop in the 50 to 60 age group, is probably not significant. With the exception of the gland of a 16-week-old fetus which showed granules in abundance, in no case under 24 years were they apparent. The cases showing granules were then grouped under the disease from which the subjects had died. Of those cases showing a large number of granules in the cortical cells, a high proportion died of acute infective conditions. An examination of suprarenals of mice and guinea pigs showed no granules in mice but granules were present in all the guinea pigs, whether male or female. It was concluded, therefore, that while age has some influence on the granules, the sex hormones play no part.

WITSCHI, E., AND G. M. RILEY. Quantitative studies on the hormones of human pituitaries. *To appear in this Journal.*

More than 100 pulvarized acetone-dried human hypophyses were assayed in various tests with the following results. No distinct variations according to age and sex were found with respect to the thyrotropic, the lactogenic and the chromatophortropic hormones. The gonadotropic hormone is present only in small quantities in children; amounts of powder below 30 mg. produced no reactions in the immature rats. In the reproductive age the hypophyses of men are about 4 times more potent than those of women. Old women (over 50) and castrates have hypophyses of highest potency. The hypophyses of old men give varied values, some of them also assuming the castrate type. During the first period of pregnancy the gland rapidly loses potency; regaining it slowly toward the end, and more rapidly after delivery. The human gonadotropic complex is unusually rich in FSH while LH is present only in traces.

ABSTRACTS OF ENDOCRINE LITERATURE

ADRENALS

Bensis, W., and A. Codounis. Paroxysmal hypertension caused by adreno-medullar tumor (Contribution a l'étude de l'hypertension paroxystique du médullo-surrénalome). *Bull. Acad. de méd.* 121: 237. 1939.

A case of neoplasm of the adrenal medulla was presented (as confirmed by autopsy) characterized by repeated crises of hypertension and hyperglycemia, but without glycosuria. The elevation of blood sugar, moderate at rest, reached extreme values during the attacks. The frequency of the latter led to cardiac instability which prevented surgical intervention.—A.E.M.

Bock, K. A. The influence of adrenal extract on the course of thyroxin induced metamorphosis in tadpoles and axolotl (Die Einwirkung von Nebennierenrindenextrakt auf den Ablauf der Thyroxinmetamorphose bei Froschlarven und beim Axolotl). *Klin. Wchnschr.* 17: 1311. 1938.

The addition of 5 cc. of cortin to a bath of 500 cc. containing 0.2 cc. of thyroxin ("1 promill.") accelerated the metamorphosis of tadpoles and axolotls beyond the rate attained with thyroxin alone. This concentration of cortin without thyroxin did not influence metamorphosis.—M. H. F.

Del Castillo, E. B., T. Schlossberg and J. L. Curuchet. Sexual function in suprarenal virilization (La fonción sexual en la virilización suprarenal). *Semana méd.* 46: 210. 1939.

A study of 80 female patients showing virilization of adrenal origin led to the following results. The menstrual cycle was affected in most cases, 26.46% showing oligomenorrhea and 39.24% secondary amenorrhea. Only 20.25% had a normal cycle. Sterility was found in 50%. Breast development was below normal in 9%, normal in 33%, excessive in 40% and extreme in 17.5%. The younger patients presented premature appearance of libido and intense orgasmus. The libido ceased earlier than the time of natural menopause. No homosexual individuals were found in the entire group.—A. E. M.

Ferrebee, J. W., C. Ragan, D. W. Atchley and R. F. Loeb. Desoxycorticosterone esters. Certain effects in the treatment of Addison's disease. *J.A.M.A.* 113: 1725. 1939.

The effects of desoxycorticosterone acetate or propionate in dosages of 10 to 25 mg. daily for 9 days, intramuscularly in oil were studied with specific reference to excretion of water, sodium, potassium, total nitrogen and riboflavin, and observations were made upon changes in hematocrit, serum volume and total interstitial fluid volume. In some cases the effects on B.M.R. and on carbohydrate metabolism were recorded. Six patients were studied. The most striking effects appeared in salt and water metabolism, retention of sodium and water, followed by improvement in patient's condition which was qualitatively identical with that following administration of salt and water alone, but quantatively more marked. No effect on carbohydrate metabolism was observed. Clinically improvement was far greater than has resulted from any therapy hitherto advocated. Caution was urged in use of esters, since excessive amounts lead to hypoproteinemia, edema and cardiac insufficiency.—H. O. H.

Fitzgerald, O., and F. Verzár. The effect of the adrenal cortical hormone on the glycogen content of the liver of hypophysectomired rats (Die Wirkung von Nebennierenrindenhormon auf den Glykogengehalt der Leber von hypophysektomierten Ratten). *Arch. f. d. ges. Physiol.* 242: 30. 1939.

The glycogen content of the liver in 16 hypophysectomized rats was markedly reduced by 8 hours starvation. Preliminary injections for 1 week with desoxycorticosterone and before the starvation period with cortical extract prevented the loss of glycogen from the liver.—A. G.

Gauer, O., and K. Kramer. The oxidation stimulating effect of adrenalin on the warm blooded animal's heart (Zur Frage der oxydationssteigernden Wirkung des Adrenaline am Warmblüterherzen). *Arch. f. d. ges. Physiol.* 242: 328. 1939.

The O_2 consumption of fibrillating and resting cat's hearts was measured by a continuous determination of the arterio-venous O_2 difference and blood flow through the coronary vessels. The coronary dilating effect of adrenalin was unaccompanied by an increased O_2 consumption. No specific stimulating effect of adrenalin on resting cardiac muscle could be demonstrated.—A. G.

Hasch, Z., and I. Hajdu. Further studies on the hypertrophy of the adrenals induced by work (Weitere Versuche ueber durch Arbeit verursachte Nebennierenhypertrophie). *Arch. f. d. ges. Physiol.* 241: 506. 1939.

Blood serum of dogs when injected into rats induced a 30% increase in weight of the adrenals. This hypertrophy was attributed to the cholesterol content of the injected blood, since injections of cholesterol solutions evoked the same response. The hypercholesterinemia attendant upon exercise in the rat is, therefore, also held responsible for the adrenal hypertrophy which occurs in this condition.—A. G.

Hermann, H. The behavior of the denervated adrenal medulla (Le comportement de la glande médullo-surrénale énervée). *Presse méd.* 46: 1554. 1938.

Adrenal denervation in the dog did not cause

histological changes in the medulla. Adrenalin still was formed, although in smaller quantities than normally, and was perfectly mobilized by electrical stimulation or by suitable pharmacological agents. It could be shown in anesthetized animals with suprarenal-jugular anastomosis that the gland secreted small quantities of a sympathico-mimetic hormone.—A. E. M.

HOFF, E. Investigations on the influence of lactoflavin and corticosterone on experimental renal diabetes (Untersuchungen über den Einfluss von Lactoflavin und Corticosteron auf den künstlichen renalen Diabetes). *Klin. Wchnschr.* 17: 1535. 1939.

The glycosuria produced by 0.75 gm. of phloridzin per kg. in dogs was diminished by repeated injections of lactoflavin just prior to and 3 hours after the phloridzin injections. Similarly the injection of 10 mg. of synthetic corticosterone, just before and 4 and 8 hours after phloridzin caused some decrease in the glycosuria, but the effect was less pronounced than in the case of lactoflavin. The author concluded that both substances increased renal tubular function, and he discusses these results in the light of the Verzár theories of the participation of lactoflavin and phosphorylation in sugar metabolism.—M. H. F.

LEWIS, J. T., AND R. O. PRIETO. Adrenal secretion in the denervated adrenal (La secreción de adrenalina por la glándula suprarrenal desnervada). *Rev. Soc. argent. de biol.* 14: 555. 1938.

The adrenals in dogs were denervated by section of the major and minor splanchnics and extirpation of the lumbar sympathetic. A few weeks later the dogs were used as donors in adrenojugular transfusion experiments; the receptors were sensitized by injection of cocaine and atropin so that they responded to 0.01 mg. adrenalin per kg. The denervated adrenal secreted more or less continuously 0.005 mg. adrenalin per kg. per min.; it could be stimulated by strong electrical stimuli applied to the surface of the gland or by plunging the electrodes into the glandular tissue; the response was significantly less than that observed in innervated glands.—J. T. L.

MOGLIA, J. A. Effect of adrenalin on potassium in plasma (Acción de la adrenalina sobre el potasio plasmático). *Rev. Soc. argent. de biol.* 15: 5. 1939.

In chloralosed dogs adrenalin was injected intravenously at a constant rate during 15 minutes. A dose of 5γ per kg. per min. produced an initial increase in plasma K lasting 5 to 10 minutes, followed by a prolonged decrease. Doses of 0.5 and 0.25γ produced a prolonged decrease without the initial increase. The larger doses were similar to the amounts secreted by the adrenals in states of emergency, the smaller ones to the 'physiological amounts of adrenalin and sympathin.—J. T. L.

TOBIN, C. E. The influence of adrenal destruction on the prenatal development of the albino rat. *Am. J. Anat.* 65: 151. 1939.

The adrenals were partially or totally destroyed in rat embryos of 17 days and older by intrauterine cauterization. Such embryos survived to term and their weights at autopsy were normal. Hyperemia was found in remaining adrenal tissue in the majority of cauterized embryos, possibly due to partial circulatory stasis produced by substances liberated from the cauterized tissues. Other than the hyperemia, there appeared no evidence of increased vascularization or of circulatory stasis in any other tissues. The pituitary, thyroid, parathyroids, thymus showed morphological changes suggestive of the change found in these glands in the postnatal adrenalectomized animal. The growth and differentiation of the gonads and other reproductive tissues which had differentiated before or after the time of cauterization were not inhibited by total or partial adrenal destruction.—*Abstract, Wistar Institute Bibliographic Service.*

WENSE, T. The demonstration of epinephrine in worms and insects (Ueber den Nachweis von Adrenalin in Würmern und Insekten). *Arch. f. d. ges. Physiol.* 241: 284. 1938.

It was possible to extract from worms and insects a substance having the biological, physical and chemical properties of epinephrin.—A. G.

ENDOCRINE GENERAL

CASTLEMAN, B., AND S. HERTY. Pituitary fibrosis with myxedema. *Arch. Path.* 27: 69. 1939.

The case reported was that of a 48-year-old Italian housewife with a pluriglandular insufficiency without indications of cachexia and diagnosed clinically as myxedematous. The postmortem observations were those characteristic of Simmonds' disease, except for absence of cachexia. Six such cases have been described in the literature.—L. B. S.

CRAMER, W., AND E. S. HORNING. Hormonal relationship between the ovary and the adrenal gland and its significance in the etiology of mammary cancer. *Lancet* 236: 192. 1939.

Adrenalectomized mice of mixed and of inbred strains were subjected to estrogen treatment, estrin being administered (dihydroprogynon) percutaneously twice weekly (0.01% solution in chloroform of the crystalline preparation). Adrenalectomy was followed by changes in the thymus, pituitary, and in the mammae which were the reverse of those produced by estrin treatment. Estrin administration exercised a protective action against the effects of adrenalectomy, as reflected in less marked loss of body weight and in growth, smaller degree of thymic and testicular atrophy, and fewer changes in the anterior pituitary and in the mammae. Survival, moreover, was prolonged.

These results were interpreted to mean that the adrenal glands possess hormonal functions which are synergic with the estrogenic activity of the ovaries. Adrenalectomy likewise was found to diminish the adverse effects of prolonged administration of estrogen. It is believed that the adrenal glands possess hormonal functions which may play a part in the etiology of mammary cancer.—L. B. S.

FRIEDMAN, M. H. F. Gastric secretion in birds. *J. Cell. & Comp. Physiol.* 13: 219. 1939.

In chickens and pigeons under nembutal anesthesia secretion of gastric juice was intermittent. Histamine caused a flow of gastric juice having a high acidity and high concentration of total chloride but a low concentration of pepsin. Acetylcholine and pilocarpine, used as substitutes for parasympathetic stimulation, each produced a gastric juice that is not only highly acid but also rich in pepsin. Adrenalin inhibited, and alcohol increased gastric secretion. The concentration of pepsin was higher in the gastric juice of the chicken than in that of the pigeon. The volume of the body fluids regulates the rate of gastric secretion.—*Abstract, Wistar Institute Bibliographic Service.*

GESSLER, C. J., J. A. HALSTED AND R. P. STETSON. Effect of estrogenic substance on the blood sugar of female diabetics after the menopause. *J. Clin. Investigation* 18: 715. 1939.

Five diabetic women who had passed the menopause were given very large injections of estradiol benzoate, 50,000 I.U. daily. In 4 cases gonadotropic substance which had been detected in urine before such therapy was not found afterward. Significant lowering of morning blood sugar levels occurred in 2 women whose onset of diabetes coincided with the menopause, and suggestive results were seen in a third whose diabetes occurred a few years after the menopause. In two others there was no effect of estrogenic treatment on the blood sugar level. In one the onset of diabetes was several years before the menopause, in the other 7 years afterward. In one case the large doses of estrogen were followed by two significant periods of uterine bleeding.—E. L. S.

MINIBECK, H. The selective absorption of sugar in cold blooded animals and the effect of adrenalectomy and hypophysectomy (Die selektive Zuckerresorption beim Kaltblüter und ihre Beeinflüssung durch Nebennieren- und Hypophysen-exstirpation). *Arch. f. d. ges. Physiol.* 242: 344. 1939.

Isotonic glucose or galactose were absorbed 2 to 3 times as rapidly from the frog's gut as were fructose, mannose, sorbose or xylose. This selectivity disappeared after adrenalectomy or hypophysectomy.—A. G.

PARSONS, S. R. Evaluation of the intradermal test for pregnancy. *Surg. Gynec. & Obst.* 68: 187. 1939.

In 679 cases, the intradermal skin test, using the technic of Gilfillan and Greeg, proved entirely unreliable. All hormonal preparations used (antuitrin-S, APL from placental tissue, progestin, antophysin, follutein) yielded equally equivocal results.—L. B. S.

SELIGSOHN, A. Cushing's syndrome and essential hypertension (Le syndrome de Cushing et hypertension essentielle). *Presse méd.* 46: 1931. 1938.

Description of a case and discussion of the endocrine interraction in which it was believed the hypertension was produced either directly by increased secretion of vasopressin or indirectly by an excess of the stimulator of the adrenal cortex. The hypertension by vasopressin subsided on medication with drugs acting upon the diencephalon, such as gardenal or aminopyrine; neither substance had an effect on the patient. An increased quantity of cortico-stimulating hormone was found in the blood. Treatment with x-ray irradiation supplemented by medication with novuril or neptal with ammonium chloride brought about general improvement, return of the blood sugar to normal values and reduction of arterial pressure.—A. E. M.

SWINYARD, C. A. Methods for volumetric determination of fresh endocrine glands. *Anat. Rec.* 74: 71. 1939.

The simple water displacement method of volumetric determination proved inaccurate for small glands. Volume could be more accurately determined by measuring the water displaced by weight with a pycnometer or indirectly by means of the Archimedes principle in which the volume was obtained by subtracting the weight of the gland in water from its weight in air. The weight-specific gravity method proved most accurate, the specific gravity determined by a modification of Hammerschlag's method for determination of specific gravity of blood, i.e., by placing the fresh gland in a glycerol-water solution of such density that the gland neither sinks nor rises to the surface when placed in the center of a column of the fluid. Specific gravity of the fluid was then measured with a pycnometer or a hydrometer of known accuracy.—*Abstract, Wistar Institute Bibliographic Service.*

THOMPSON, H. E., AND W. T. POMMERENKE. Electrolyte and nitrogen balance in pregnancy. *J. Nutrition* 17: 383. 1939.

Electrolyte and nitrogen balance studies were conducted under practically constant supervision on two women in the 4th, 8th and 9th months of gestation. The same diet was used in each experiment and was of constant composition from day to

day. Disregarding losses through the skin, the average daily retentions of Na and K based on the 3 metabolic studies were observed to be 14.7 m. eq. and 7.9 m. eq. respectively. Fluctuations in potassium retention varied less from day to day than did those of Na and Cl. Variations in Na and Cl retention appeared to parallel one another. Such a parallelism did not seem to apply to K. The subjects were maintained in approximate chloride equilibrium. For only 1 experiment was a positive nitrogen balance observed. The nitrogen deficit in one of the experiments may be explained on the basis of diarrhea. The need of prolonged periods of continuous observation was stressed.—*Abstract, Wistar Institute Bibliographic Service.*

WINKLER, H., AND A. BINDER. The influence of experimental scurvy on the histologic structure of the hypophysis, ovary and adrenal in guinea pigs (Der Einfluss des experimentellen Skorbuts auf die histologische Struktur der Hypophyse, des Ovarium und der Nebenniere beim Meerschweinchen). *Zentralbl. f. Gynäk.* 63: 2114. 1939.

In immature and adult guinea pigs scurvy was induced by dietary limitations. The pituitary showed slight changes, with increased intercellular substance, and changes toward less eosinophils. Primordial ovarian follicles were reduced, and ova in larger follicles showed degeneration. Marked adrenal cortical degeneration was seen. Ascorbic acid content of these glands was almost nil.—E. L. S.

GONADS

AIELLO, R. J. The action of corpus luteum hormone on the gravid hypophysectomized bitch (Acción de la hormona del cuerpo amarillo en la perra grávida e hipofisectomizada). *Semana méd.* 45: 1400. 1938.

Injections of 10 mg. of synthetic progesterone and of natural progesterone delayed abortion following hypophysectomy in the pregnant bitch.—A. E. M.

BIDDULPH, C. The effect of testosterone propionate on gonadal development and gonadotropic hormone secretion in young male rats. *Anat. Rec.* 73: 417. 1939.

The pituitary glands of 11 males injected from birth with 10γ of testosterone propionate failed to show castration hypersecretion following gonadal removal, as tested by parabiosis, whereas the pituitaries of 11 normal and of 9 injected with 2γ did undergo hypersecretion, similarly tested. Normal testicular growth was prevented by injection of 2 to 50γ of testosterone propionate from birth to 31 to 81 days. Injury of seminiferous tubules and delay in time of sperm appearance occurred with the larger doses. Treated rats had smaller accessory glands than the controls at every autopsy age except in those injected for 31 days with 10γ per day, and in animals allowed to survive discontinuance of treatment the testes and accessories did not attain the same weight as the corresponding glands in normal animals. Maintenance of normal accessories required higher dosages than were required to prevent gonadotropic secretion. Accessories and interstitial tissue of 8 injected animals were greatly stimulated following subsequent injection of pituitary extract. Testis descent was hastened with daily doses of 2γ and prevented with 10 to 50γ for periods up to 55 and 60 days beyond normal time of descent. The scrotal area of injected rats was not so well developed as that of normal animals.—*Abstract, Wistar Institute Bibliographic Service.*

BISKIND, G. R., AND J. MARK. The inactivation of testosterone propionate and estrone in rats. *Bull. Johns Hopkins Hosp.* 65: 212. 1939.

Pellets of testosterone propionate or estrone failed to exert any effects in castrate rats when implanted in the spleen. The specific effect returned when the spleen containing the pellets was transplanted and the splenic artery and vein were ligated.—A. G.

BRINDEAU, A., H. HINGLAIS AND M. HINGLAIS. The gonadotropic secretion of the placenta and its functional activity (Secretion gonadotrope et activité fonctionelle placentaire). *Presse méd.* 47: 281. 1939.

Titration of the luteinizing action of the prolan B contained in the serum gave a measure of the activity of placental tissue. Using RB. U., the following scale is presented. 1. Non-pregnant women, zone of uncertainty, 0 to 150 units. 2. Pregnant women with placental insufficiency and weakness, with fetal death, 0 to 500 units. Weakness, but with no certainty of death of fetus, 500 to 2000 units. Normal placenta, 2000 to 25,000 units. Hyperactive placenta, toxic pregnancy, 25,000 to 60,000 units. Hydatiform mole, 60,000 and more.—A. E. M.

BURGER, J. W. Some experiments on the relation of the external environment to the spermatogenetic cycle of Fundulus heteroclitus (L). *Biol. Bull.* 77: 96. 1939.

Spermatogenetic cycles were not affected by light but the temperature of the water proved an important factor. Sexual activity was inhibited near 0° C. and at 10° C. spermatogonial multiplication occurred but higher temperatures were required for complete spermatogenesis.—E. L.

BURNS, R. K., JR. The differentiation of sex in the opossum (Didelphys virginiana) and its modification by the male hormone testosterone propionate. *J. Morphol.* 65: 79. 1939.

The young of the American opossum are very immature at birth. Sex differentiation is beginning in the gonads but dimorphism in other parts of

the reproductive apparatus does not occur until much later. Treatment with crystalline sex hormones during the first weeks of pouch life produced striking effects upon the differentiating parts of the reproductive system. Testosterone propionate produced enormous hypertrophy of the phallus in both sexes, with quantitatively comparable results. Scrotum and marsupium were apparently unaffected. Wolffian ducts differentiated precociously into vasa deferentia and epididymides in both sexes, and rete connections were present. Prostates hypertrophied and bulbourethral glands proliferated. However, the hormone likewise induced development of all müllerian duct derivatives, especially in females, uterine development in males was marked and sufficient to arrest descent of the testes, but vaginas were usually absent. Testosterone propionate behaved, therefore, both as a male and female hormone. Its androgenic activity in the male subject was, however, greater quantitatively than its effect upon homologous parts in females. Conversely its effect upon female parts was relatively greater in the female subject. A constitutional sex factor appeared to limit these effects in a quantative way, but not qualitatively. Differentiation of gonads was apparently unmodified.—*Abstract, Wistar Institute Bibliographic Service.*

CHAMPY, C., H. BOYER AND R. COUJARD. The mechanism of action of male sex hormones in prostatic hypertrophy (Le mécanisme de l'action des hormones males sur l'hypertrophie prostatique). *Presse méd.* 46: 1097. 1938.

Purified testicular lipids were found preferable to testosterone since, in equivalent unit doses they proved more efficacious in treatment of perivascular edema of the prostate. The clinical improvement consisted in a decrease of the vesicular residue and a facilitation of urination. A reduction of the adenoma was not demonstrated. From animal experiments it was concluded that the prostatic edema is caused by relaxation of the smooth muscle fibers and that a return of the tonus is the first effect produced by the sex hormones.—A. E. M.

COBET, R., M. RATSCHOW AND M. L. STECKNER. Experimental investigations on the effect of diethyl-dioxystilbene (Cyren) on the peripheral blood supply (Experimentelle Untersuchungen über die Wirkung eines synthetischen Follikulin ersatzstoffes Diäthyl-dioxystilben (Cyren) auf die periphere Blutversorgung). *Klin. Wchnschr.* 18: 278. 1939.

The injection of 0.5 mg. of cyren dipropionate for 6 days before and for 6 days after injection of 3.5 to 4.4 mg. of ergotamine tartrate protected female rats against tail necrosis. Similar results were obtained with the diacetate. In contrast to estrone, the protection afforded by these compounds was found to be as great in males as in females. In males and females admixture of the ergotamine with 0.0005 c.c. of 1/1000 adrenalin produced a very severe tail necrosis, against which estrone was ineffective. However, injection of 0.5 mg. of cyren diacetate for 2 days before and for 2 days after the injection of the ergotamine-adrenalin mixture afforded complete protection.—M. H. F.

VEST, S. A., AND J. E. HOWARD. Clinical experiments with androgens. IV. A method of implantation of crystalline testosterone. *J.A.M.A.* 113: 1869. 1939.

By means of a specially devised injector, described in detail, 13 patients with hypogonadism were implanted with pure testosterone pellets subcutaneously or intramuscularly. Two case histories were included, illustrating beneficial effects of testosterone administration in hypogonadism. Local tissue reaction to pellet implantation was described with emphasis upon the fact that no unusual reaction developed.—H. O. H.

VIGNES, H. The effect of zinc in various conditions of the uterus and ovaries (Action du zinc sur divers états utéro-ovariens). *Bull. Acad. de méd.* 121: 412. 1939.

In view of the fact that zinc alters the action of some hormones by increasing their effect, the influence of zinc medication in utero-ovarian disturbances was studied. The acetate or valerianate was given in doses of 100 mg. daily. The 75 cases of menstrual disorders, especially menorrhagia and amenorrhea treated showed a high incidence of improvement. One of 2 cases of dysmenorrhea improved. Cessation of treatment was usually followed by a relapse. Five cases of threatened abortion were brought to term, but other treatment was used simultaneously. Among 22 cases of sterility 7 were brought to pregnancy.—A. E. M.

HYPOPHYSIS

KUSCHINSKY, G., AND P. LIEBERT. Investigations of the hormone content of the posterior pituitary of the rat under the influence of water, salt, and novasurol (Untersuchungen über den Hormonegehalt des Hypophysenhinterlappens der Ratter unter dem Einflusz von Wasser, Kochsalz un Novasurol), *Klin. Wchnschr.* 18: 823. 1939.

Pituitaries removed 24 hours after the last of a series of injections of 5 cc. of water per 100 gm. body weight showed an anti-diuretic and an oxytocic hormone content reduced to about ½ the control level (details of technic not given). However, administration of a single dose of 5 cc. water per 100 gm. body weight led to an increase in antidiuretic material in glands removed 24 hours later. Administration of 10 mg./kg. of novasurol led to a decrease, but after 15 mg. per kg. of novasurol plus 5 cc. of water per 100 gm. body

weight there was an increase of the antidiuretic content.—M. H. F.

LARSON, E. Fate of the injected oxytocic principle of posterior pituitary in anesthetized cats and dogs. *J. Pharmacol. & Exper. Therap.* 67: 175. 1939.

Post-pituitary solution U.S.P., 3/5 or 1 3/5 cc. per kg. of body weight was injected intravenously into anesthetized cats or dogs. Urine eliminated, following these injections, contained 30% or less of the injected oxytocic principle, the greater portion being present in samples collected during the first 15 minutes. Kidney and liver tissues readily inactivate the oxytocic principle. From studies with purified enzyme preparations, the effects of various PH concentrations and the effects of certain enzyme inhibitors, the author concluded that the oxytocic principle can be inactivated by tissue polypetidases.—E. L.

MAGRUDER, S. R. Unusual reactions of gastric mucosa following hypophysectomy. *Anat. Rec.* 73: 269. 1939.

A group of F_2 Dane-St. Bernard pups that had been hypophysectomized showed an enormous increase in the lymphatic tissue of the gastric mucosa. Certain striking histological changes in the liver occurred, as evidenced by congestion and by depletion of glycogen. Such changes occurred in a comparatively short time as there is 100% mortality in this group within 16 days after operation. Although a large number of dogs from various breeds have been hypophysectomized, the post-operative mortality has been low and only these F_2 Dane-St. Bernard pups have shown 100% mortality or exhibited the condition of the gastric mucosa described.—*Abstract, Wistar Institute Bibliographic Service.*

MOSONYI, J. Hypophysectomy in the dog (Hypophysenexstripation beim Hunden). *Arch. f. d. ges. Physiol.* 242: 92. 1939.

By placing the dog in a vertical position hypophysectomy could be performed by the buccal approach without hemorrhage or loss of cerebrospinal fluid.—A. G.

STEAD, E. A., JR., P. KUNKEL AND S. WEISS. Effect of pitressin in circulatory collapse induced by sodium nitrite. *J. Clin. Investigation* 18: 673. 1939.

Pitressin in man was found ineffective in experimentally induced collapse (sodium nitrite and tilting) because it did not cause an increase in venous tone, and because the arteriolar constriction produced by the drug tended further to reduce the tissue blood flow. The intramuscular injection of 1 cc. of pitressin with subsequent tilting produced collapse ending in syncope in 2 of 6 normals. Pitressin failed to prevent collapse from nitrite plus tilting, and in 2 cases seemed to increase the collapse reaction from nitrite. Pitressin caused abdominal cramps, ashen pallor and decreased blood flow in the hand at 32 or 43° C. It produced no change in venous tone in the hand, in heart rate or arterial pressure. Pitressin slowed the blood flow in the hand.—E. L. S.

ZONDEK, H., AND A. KATZ. Pituitary diabetes: the effect of fever (Diabète hypophysaire: action de la fièvre). *Presse méd.* 46: 1835. 1938.

In two cases described pituitary pathology was evident in the first patient by obesity and psychic depression associated with diabetes. The other patient had a pituitary tumor with signs of pituitary dissociation, dwarfism, akromicria, hypergenitalism, osteoporosis, retardation of ossification, psychic infantilism and latent diabetes. Fever produced in both patients a remarkable increase in sugar tolerance. A mechanism involving the adrenal cortex is supposed to explain the observations.—A. E. M.

PANCREAS

BAISSET, A., L. BUGNARD, C. DARNAUD AND C. SOULA. A study of the hepatic circulation following insulin injection (Étude de la traversée hepatiques après une injection d'insuline). *J. de physiol. et de path. gen.* 37: 592. 1939.

Simultaneous blood samples from the hepatic, portal and femoral or saphenous veins of 6 normal unesthetized dogs were analyzed for blood sugar by the Folin-Malmross method before and after injection of a sub-convulsive dose of insulin. The level in hepatic venous blood was higher than that in portal blood, and the latter higher than femoral or saphenous venous blood values throughout the duration of the experiment, except in 2 cases in which the relationship was observed only after 2 hours. The observations were taken to indicate that the site of action of insulin in producing hypoglycemia is in tissues other than the liver. The liver was believed to act antagonistically toward insulin, releasing a maximum amount of sugar into the blood in the period during which the blood sugar level is at its minimum.—Kermit Pines.

BEARDWOOD, J. T., JR. AND J. W. CARMUTH. Two cases of attempted suicide with protamine-zinc insulin. *Internat. Clin.* 3: 240. 1939.

The cases are reported because of their rarity and to illustrate the importance of the administration of tremendous doses of glucose in treatment. Both patients were psychoneurotic individuals. It was not until hours after the blood sugar had been kept at a high level that consciousness was restored.—I. B.

BOWEN, B. D., J. S. REGAN AND E. C. KOENIG. The development of arteriosclerosis in the diabetic, based on the study of a group of patients during ten to thirteen years. *Ann. Int. Med.* 12: 1996. 1939.

Proof is offered that arteriosclerosis of the lower extremities can be prevented or delayed by controlling the diabetes continuously provided the control is started early enough after the inception of the disease.

BURKE, J. C., AND A. R. MCINTYRE. Nicotinic acid and the vitamin B complex in insulin tolerance. *J. Pharmacol. & Exper. Therap.* 67: 142. 1939.

Groups of rats, on a vitamin-free diet supplemented with thiamin hydrochloride, were fed nicotinic acid, vitamin B_6 or lipid material from rice polishings. Insulin tolerance was determined after the administration of 1γ crystalline insulin per 10 gm. of body weight. Crystalline B_6 did not prevent the augmented response to insulin produced by thiamin but nicotinic acid and the lipid material decreased the duration of hypoglycemia. —E. L.

BUZZO, A., AND R. CARRATALÁ. Insulin intoxication (La intocicación por insulina). *Semana méd.* 46: 96. 1939.

Eight commercial samples of insulin were found to be widely at variance with the declared strength which fact accounted for the variable degrees of toxicity observed. Prolonged treatment with small doses did not lead to an increased resistance of animals, but produced psychic disturbances.—A. E. M.

DUFF, G. L. The clinical and pathological features of carcinoma of the body and tail of the pancreas. *Bull. Johns Hopkins Hospital.* 65: 69. 1939.

Sixteen unselected cases of primary carcinoma of the body or tail of the pancreas were analyzed from clinical and pathological points of view. Such cancers are frequently accompanied by ascites, hematemesis and enlargement of the spleen or liver. These symptoms result from the anatomical relations of the body and tail which lead to direct extension of the carcinoma to the peritoneum, stomach, intestines, or invasion of the splenic vein with metastases to the liver.—A. G.

HEMMINGSEN, A. M. Improvements in the accuracy of insulin assay on white mice. *Skandinav. Arch. f. Physiol.* 82: 105. 1939.

The mortality of mice used in the assay of insulin was reduced from 12 to 0.3% by maintaining the animals at 25° C. at all times. The standard deviation of a test utilizing 160 mice was reduced from 12 to 25% to 7 to 10% by retaining the same animals in each grouping.—A. G.

MALTESOS, C., AND R. H. WATSON. Blood flow and secretion of the pancreas during humoral stimulation (Durchblutung und Sekretion des Pankreas bei humoraler Anregung). *Arch. f. d. ges. Physiol.* 241: 516. 1939.

The volume of blood flow and the volume of secretion from the pancreas were found to increase proportionately when the gland was stimulated by HCl or by secretin.—A. G.

MILLER, M. M., AND O. P. ALLEN. A new mathematical method for the evaluation of endogenous insulin secretion. *Ann. Int. Med.* 13: 636. 1939.

A method has been worked out for computing the dosage of insulin required to lower the blood sugar to any desired level.

PARKES, A. S., AND F. G. YOUNG. The influence of the subcutaneous implantation of tablets of solid insulin on the blood sugar level of the rabbit. *J. Endocrinol.* 1: 108. 1939.

Tablets of crystalline or low zinc insulin weighing 5 mg. were implanted into the shaved flanks of rabbits weighing 1.5 to 2.0 kg., under local anesthesia. In some cases aseptic precautions were taken and in these instances the insulin was sterilized by heating it to 100° C. for 1 hour on each of 3 successive days. This resulted in some inactivation, but the crystalline insulin was less affected than the low zinc material. It was found that these tablets (which contain approximately 100 U of insulin) had a hypoglycemic activity which was only slightly more prolonged than 100 U of subcutaneously administered solution. Heat sterilized tablets were similar to unsterilized tablets. It was found that tablets of low zinc insulin were completely absorbed within 24 hours of implantation. With crystalline material a capsule containing crystals of insulin formed at the site of implantation. In spite of this there was little difference in the effectiveness of the 2 preparations. It is suggested that part of the absorbed insulin had been inactivated or excreted.—G. B.

POLLACK, H., AND H. DOLGER. Advantages of prozinsulin (protamine zinc insulin) therapy: dietary suggestions and notes on the management of cases. *Ann. Int. Med.* 12: 2010. 1939.

With proper dietary control, prozinsulin offers advantages for the routine care of diabetic patients. Among them are, a single daily injection, elimination of ketonuria, approximation to normal carbohydrate metabolism, and decrease in the incidence of hypoglycemic episodes.—From authors' summary.

RABINOWITCH, I. M. The significance of post-prandial glycosuria in the treatment of diabetes mellitus with protamine zinc insulin. *Ann. Int. Med.* 13: 385. 1939.

The conclusion, that the post-prandial glycosuria in diabetes treated with protamine zinc insulin, when associated with a perfectly normal blood sugar in the fasting state, is not harmful. This conclusion is based entirely upon use of protamine zinc insulin with our high carbohydrate-

low calorie diet. It may or may not apply to other diets.—From authors' summary.

SECKEL, H. P. G. Postmortem hepatic glycogenolysis in hyperinsulinism and glycogen disease. *J. Clin. Investigation* 18: 723. 1939.

In 2 adult cases of spontaneous hypoglycemia, the one probably due to pancreatogenic hyperinsulinism (carcinoma of pancreas with liver metastases), the other possibly due to neurogenic hyperinsulinism (massive fibroma on top of right liver lobe), there was demonstrated a comparatively high liver and muscle glycogen content with approximately normal postmortem hepatic glycogenolysis. Since typical cases of glycogen disease (Von Gierke) are characterized by abundance of glycogen but inhibition of postmortem glycogenolysis in liver, it is held that typical glycogen disease cannot arise from either of these forms of hyperinsulinism.—E. L. S.

VOIGHT, W. W. Diabetes and surgery. *Illinois M. J.* 76: 167. 1939.

This paper is based upon the lesson drawn from a case history. The patient succumbed despite the cooperation of surgeon, internist and roentgenologist. In cases of gangrene, insulin treatment is not usually successful because the major factor in the emergency is not the diabetes but rather the arteriosclerosis and thrombosis. However, in infectious processes complicating diabetes, insulin treatment is highly efficacious in the surgical management of the case. In all cases the general management of the patient must be carried out in accordance with the current conceptions of treatment.—I. B.

PARATHYROIDS

LOGAN, M. A. The early effects of parathyroid hormone on the blood and urine. *J. Biol. Chem.* 127: 711. 1939.

The changes in blood levels and urinary excretion were determined at hourly intervals in 5 dogs that had been fasted for 3 to 5 days and then injected with parathormone. It was found that the urinary phosphate excretion increased and the plasma inorganic phosphate decreased during the first hour. This would indicate a direct effect of the hormone on the kidney. At the same time there was an increase in blood calcium which was explained as the effect of active solution of bone by the parathormone.—J. M. L.

ROSE, E., AND F. W. SUNDERMAN. Effect of dihydrotachysterol in treatment of parathyroid deficiency. *Arch. Int. Med.* 64: 217. 1939.

Observations are reported on 5 patients, with parathyroid deficiency following thyroidectomy, who were treated with dihydrotachysterol. These observations included determinations of calcium balance, total and diffusible serum calcium, serum protein and inorganic serum phosphorus before and during the administration of dihydrotachysterol. Dihydrotachysterol was found highly effective in increasing the concentration of serum calcium and in relieving the symptoms of parathyroid deficiency. The authors find that in excessive doses it is capable of producing hypercalcemia with toxic symptoms. The diffusible and the nondiffusible fraction of the serum calcium share about equally in the rise in concentration of total serum calcium which follows the administration of dihydrotachysterol. Insofar as the data permit conclusions, the trend of changes in the urinary and the fecal calcium in this series of cases supports previous opinion that dihydrotachysterol increases urinary excretion of calcium at the expense of fecal calcium.—I. B.

THYROID

ALTHAUSEN, T. L., W. J. KERR AND M. STOCKHOLM. Calcium exchange of the intestine in experimental hyperthyroidism. *J. Clin. Investigation* 18: 763. 1939.

Fecal excretion of calcium by rats was approximately doubled by thyroxin or sub-purgative doses of castor oil or cascara. Excretion was decreased in hyperthyroid rats by restricting food intake or use of morphine. The authors feel they have ruled out as causes for increased fecal calcium loss increased permeability of the intestinal mucosa, active excretion of calcium, or increased absorption of other foods. The increased fecal output of calcium is attributed to overeating and hyperperistalsis. There was no appreciable alteration in calcium absorption in rat hyperthyroidism.—E. L. S.

CARRIÈRE, C., J. MOREL AND P. GINESTE. The action of vitamin excess and of deficiency diets on the thyroid (Action des hyperviamines et des régimes carencés sur la glande thyroïde). *Bull. Acad. de med.* 121: 324. 1939.

Daily doses of 10,000 to 25,000 u of vitamin A and 50 to 100 mg. ascorbic acid given subcutaneously inhibited the activity of the thyroid in the rabbit and finally led to a gland resembling in structure the thyroid in colloid goiter. Similar effects were observed in rats. Vitamin B_1 in doses of 10 to 20 mg. per day caused in guinea pigs a moderate stimulation. The vesicular epithelium became cylindric and hypertrophic. Several mg. of vitamin D produced in the rabbit intense hyperplasia with numerous mitoses. Prolongation of this treatment led finally to degeneration of the gland. Deficiency of A led in the rat to histological signs of hypersecretion, with gradual decrease in colloid and ending in desquamation of the epithelial elements and atrophy. C deficiency in guinea pigs was associated with hyperactivity of the thyroid. It was concluded that A and C are helpful in hyperthyroidism but that D and helio-

therapy are contraindicated; the latter may be of value in hypothyroidism. Clinical observations seem to support these findings.—A. E. M.

CUTTING, W. C. AND G. B. ROBSON. Effects of various agents on the metabolic rate in experimental hyperthyroidism. *J. Pharmacol. & Exper. Therap.* 66: 389. 1939.

Metabolic rate determinations were made weekly on 29 guinea pigs in which experimental hyperthyroidism had been induced by pituitary thyrotropic hormone. After administration of the following drugs:—sodium fluoride, vitamins A, D, B_1 or B_2, sodium iodide, estradiol, ergotamine, quinine hydrochloride, cevitamic acid and sodium thiocyanate—the metabolic rate was redetermined. Only sodium iodide caused any appreciable decrease in the metabolic rate.—E. L.

DEL CASTILLO, E. B., AND J. R. MEMBRIVES. Aspects of pathogenesis of hyperthyroidism (Aspectos de la patogenia del hipertiroidismo). *Semana méd.* 46: 1452. 1939.

Two cases of typical hyperthyroidism were described which after operative treatment showed improvement so far as cardiovascular disturbances, body weight and metabolism were concerned. Simultaneously the nervous symptoms of hypothalamic origin showed exacerbation. Exophthalmos became more accentuated and mucoid patches appeared symmetrically on both legs. The usual classification of the latter as circumscript myxedema was rejected, and a hypothalamic nervous origin postulated. The patients were siblings. It was concluded that hyperthyroidism is a syndrome involving nervous as well as endocrine factors.—A. E. M.

DUCOURT, J. The thyroid and the regulation of blood chlorides (Le rôle du corps thyroïde dans la régulation de la chlorémie). *Ann. méd.* 44: 133. 1939.

Hyperthyroidism tended to cause an increase in blood chloride, myxedema to decrease it. The chlorine content in 14 patients with Basedow's disease varied from 2.17 to 2.37 in the corpuscles and from 3.92 to 4.11 in the plasma. The corresponding figures in 7 myxedematous patients were 1.46 to 1.70 in cells and 3.04 to 3.47 in the plasma. Normal persons showed the maxima of 2.05 and 3.88 respectively, minima of 1.77 and 3.55. Thyroid treatment of myxedema caused an increase in blood chlorine as the metabolism approached normal values. Thyroid-treated rabbits showed an analogous increase in chlorine. The changes were always more marked in the corpuscles than in the plasma.—A. E. M.

GILDEA, E. F., E. B. MAN AND J. P. PETERS. Serum lipoids and proteins in hypothyroidism. *J. Clin. Investigation* 18: 739. 1939.

Study of serum cholesterol, protein, and basal metabolism was correlated with clinical findings and results of therapy in 29 patients with myxedema or suspicion of hypothyroidism. Patients with myxedema had remarkably high serum lipoids, and amelioration of symptoms by 1 to 2 grains of U.S.P. thyroid was closely paralleled by fall in lipoids and rise in basal rate. The changes were reversible. In patients without hypothyroidism (poor response to therapy) the changes in serum cholesterol were not so significant. Therefore the serum cholesterol is a useful tool in determining the presence or absence of hypothyroidism. Serum cholesterol below 275 mg. per 100 cc. practically excludes the diagnosis of hypothyroidism and indicates that thyroid therapy will have little effect in relieving symptoms which resemble those of myxedema. Serum proteins tend to be high in hypothyroidism, but the levels cannot be consistently correlated with the amount of edema, due probably to complicating nutritional and metabolic factors.—E. L. S.

GINSBURG, S. Postoperative progressive exophthalmos with low basal metabolic rate. *Ann. Int. Med.* 13: 424. 1939.

The favorable results of roentgen-ray treatment of the orbital and pituitary regions in cases of postoperative progressive exophthalmos with low B.M.R. suggest the early use of radiation therapy in combination with medical treatment for exophthalmos in Graves' disease whenever it becomes a distinct clinical problem.—From author's summary.

KING, F. H., AND A. R. SOHVAL. The relative value of the basal metabolic rate, velocity of blood flow and creatine tolerance test in the differential diagnosis of Graves' disease and allied conditions. *Ann. Int. Med.* 13: 261. 1939.

Clinical judgment surpassed in value laboratory aids. Determination of basal metabolic rate excels the circulation time test and creatine tolerance test in usefulness.—From authors' summary.

PERKIN, H. J., AND L. M. HURXTHAL. The fractionation of the iodine of the blood in thyroid disease. *J. Clin. Investigation* 18: 733. 1939.

Using a method devised for the purpose the organic iodine was found decreased in blood of 10 patients with myxedema. There was an increase in organic iodine in 98 patients with exophthalmic goiter, before iodine therapy, decreasing after use of iodine. Patients (7) with non-toxic goiter receiving iodine had similar organic iodine levels to the exophthalmic patients on iodine, but had somewhat less inorganic iodine. The normals employed were 65 patients with non-toxic goitre, not receiving iodine.—E. L. S.

PERKIN, H. J., AND R. B. CATTELL. Blood iodine levels related to the recurrence of hyperthyroidism. *Surg. Gynec. & Obst.* 68: 744. 1939.

In 256 cases of exophthalmic goiter 833 blood iodine determinations were made. None of the patients had received treatment prior to coming under observation, and of the number 100 were followed for 1 year. Hyperthyroid patients with an elevated pre-operative blood iodine showed a normal blood iodine following subtotal thyroidectomy. In the majority of patients with hyperthyroid symptoms of a year's duration, the iodine level was normal, but following subtotal thyroidectomy an increase occurred, with an incidence of recurrence of 19.7%. If symptoms had been present for 4 to 5 years, the blood iodine was generally normal and was uninfluenced by subtotal thyroid removal. Evidence is presented to show that the pre-operative blood iodine level can be used as an index of the amount of gland to be removed in hyperthyroid patients. Radical subtotal thyroidectomy is recommended in all cases of hyperthyroidism with a normal or a low pre-operative blood iodine level.—L. B. S.

Sharpless, G. R., Janice Pearsons and Geneva Prato. Production of goiter in rats with raw and with treated soy bean flour. *J. Nutrition* 17: 545. 1939.

A diet composed of unprocessed soy bean flour, yeast, sugar, butterfat, sodium chloride and calcium carbonate, when fed to rats, produced thyroid enlargement of 4 times normal in an experimental period of 7 weeks. Better growth was obtained when butterfat and yeast were present, but they were not essential parts of the diet. Even though the iodine content was greater, a larger thyroid was obtained when the diet contained 75% soy bean flour than when it contained 35%. the minimum iodine requirement of rats fed the soy bean flour was twice normal or 50 gamma per 1000 calories. The optimum iodine requirement of approximately 200 gamma per 1000 calories was also twice normal. When the iodine content was unchanged, soy bean flour which had been steamed or extracted with fat solvents (ether or acetone) did not produce so large a gland as untreated flour. It was concluded that unprocessed soy bean flour possesses a positive goiterogenic property.—*Abstract, Wistar Institute Bibliographic Service.*

Smelser, G. K. Testicular function and the action of gonadotropic and male hormones in hyperthyroid rats. *Anat. Rec.* 73: 273. 1939.

Administration of thyroxin or dried thyroid gland to adult males caused a decrease in testis weight, sperm production, and accessory weight. In amounts too small to effect weight loss, thyroxin affected testicular activity in both adult and immature males; with larger doses more marked damage to the reproductive system occurred. Gonadotropic preparations could maintain normal testicular function and accessory structure in hyperthyroid rats, although in immature hyperthyroid males their effectiveness was markedly decreased. The amount of testis hormone required to stimulate accessories of castrates was increased approximately 5 times in thyroxin treated animals. No evidence was obtained to indicate either a subnormal or abnormal production, secretion or destruction of gonadotropic hormones in the hyperthyroid animals. Hypofunction of the male reproductive tract in hyperthyroidism may be explained by an increase in the threshold of response to gonadotropic and male hormones.—*Abstract, Wistar Institute Bibliographic Service.*

Smelser, G. K. The effect of thyroidectomy on the reproductive system and hypophysis of the adult male rat. *Anat. Rec.* 74: 7. 1939.

Nineteen litters of adult males were thyroidectomized for periods varying from 41 to 200 days. One animal in each litter was reserved as a control. Thyroidectomy caused a reduction in accessory weight and sperm production thus indicating a decrease in both the germinal and endocrine phases of testicular activity. The gonadotropic hormone content of the hypophysis was not affected by thyroidectomy even though a definite increase in weight of the pituitary gland occurred and a great number of large basophilic 'signet ring' cells were formed.—*Abstract, Wistar Institute Bibliographic Service.*

Thorner, M. W. Relation of myasthenia gravis to hyperthyroidism. *Arch. Int. Med.* 64: 330. 1939.

A case of myasthenia gravis is presented in which the appearance of hyperthyroid symptoms was attended by a lessening of the myasthenic symptoms. The administration of thyroid substance to a patient with uncomplicated myasthenia gravis coincided with a period of remission of the myasthenic symptoms. In 2 cases previously reported by others there was some evidence of this same type of 'seesaw' balance between myasthenia gravis and exophthalmic goiter. For these reasons the author suggests that the 2 diseases are mutually antagonistic. It is felt that this relation is a matter of scientific but not of immediate therapeutic importance.—I. B.